LIVER INJURY

Transactions of the Ninth Conference
April 27 and 28, 1950, New York, New York

Edited by

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DEPARTMENT OF MEDICINE
UNIVERSITY OF MINNESOTA MEDICAL SCHOOL
MINNEAPOLIS 2, MINNESOTA

Sponsored by the
JOSIAH MACY, JR FOUNDATION
565 Park Avenue, New York, N Y

Price $3.00

Printed in the United States of America
by Corlies, Macy & Company, Inc., New York, N. Y.

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Ninth Conference On Liver Injury

JOSIAH MACY, JR. FOUNDATION CONFERENCE PROGRAM

FRANK FREMONT-SMITH

Medical Director

I WANT to welcome you to this Ninth Conference on Liver Injury. For the benefit of the guests present may I take a few minutes to explain the nature and purposes of the Foundation's Conference Program.

You have been brought together to exchange ideas, experiences, data, and methods in an effort to further knowledge in this field. However, the Foundation is also interested in investigating the broad aspects of the problem of communication and integration. Experience gained from many research projects presented for consideration has led to the conviction that one of the greatest needs today is a reintegration of science, which at the present time is artificially fragmented by the isolation of the several disciplines or specialties. We feel that the setting up of physiological and—what is probably more important—psychological barriers between the several branches of science is seriously interfering with scientific progress. Although the fertility of the multiprofessional approach is recognized, adequate channels of interprofessional communication do not exist. The Conference Program hopes to encourage this reintegration.

Thirteen conferences are now in operation covering the following fields: aging, adrenal cortex, biological antioxidants, blood clotting and allied problems, connective tissues, cybernetics, factors regulating blood pressure, infancy and childhood, liver injury, metabolic interrelations, nerve impulse, problems of consciousness, and renal function. Each of these conference groups meets annually over a period of five years for two days of informal discussion.

When a new conference is organized, fifteen scientists are selected by the Chairman in consultation with the Foundation to be the original members. Every effort is made to include representatives from all pertinent disciplines, but, for purposes of promoting full and free participation of all members and guests, attendance at any meeting is limited to a total of twenty-five.

In contradistinction to the usual scientific meeting we like to have the presentations relatively brief, and to stress the difficulties encountered rather than to report neat solutions of problems. The introductory presentations at our conferences are merely the launching of the ship — the voyage is the important thing! In other words, we feel that the heart of these meetings is in the discussion. Therefore we hope you will not hesitate to speak spontaneously and informally. What you say may not be too wise — perhaps even foolish — but how can you be sure it will not evoke wisdom from someone else!

One point which should be stressed is that between the disciplines *there are real difficulties in communication* — *partly emotional and* partly semantic. Emotionally some investigators accept only data derived from methods or disciplines with which they are familiar. On the semantic level the physical and biological sciences can understand each other without difficulty as can the medical, psychiatric, and social sciences However, to bridge the gap between the physical and biological sciences on the one hand and the psychological and social sciences on the other is very difficult. Through the Conference Program and in the published transactions this Foundation hopes to give a clearer reproduction of what takes *place in the laboratory and what goes on in the minds of scientists* than now appears in the scientific literature.

This program is an experiment and you are part of that experiment. The success of the undertaking is measured entirely by what each participant gains from this experience. We hope that here you will feel the freedom inherent in the scientific method and that you will help us to improve our conference procedure.

SECTION I

CORRELATION OF HEPATIC FUNCTION AND STRUCTURE BASED ON LIVER BIOPSY STUDIES*

HANS POPPER

The Hektoen Institute for Medical Research, Cook County Hospital

THE PROBLEM of liver biopsy is extremely difficult for the pathologist because he has to learn a new pathology of the liver I believe the greater part of what is to be learned is not known yet, and I think I will have to follow the suggestion of Dr. Fremont-Smith to present an unfinished package. I plan to present two aspects of the problem The first deals with the correlation of the histologic changes in the liver with functional alterations, the second, with a few histochemical data, specifically pentose nucleic acid in the liver.

We feel that in the whole approach to liver biopsy the principal controls, otherwise used in biologic studies, have often not been applied We have described certain pictures in some diseases but we are not yet sure that these findings are characteristic and diagnostic of the disease I want to exclude some aspects right at the beginning I am sure Dr Schiff will discuss some of them, for instance, the value of liver biopsy in the diagnosis of diseases not primarily hepatic I would rather concentrate on basic aspects

The first problem is the difference between the picture in the biopsy specimen and in the liver obtained after death(1) To use an actual example, in a liver biopsy specimen from a woman with a mild toxic hepatitis, the liver cell plates showed the usual arrangement the cytoplasm of the cells was vacuolated due to the presence of glycogen and the interstitial tissue spaces between the liver cells and the sinusoids were hardly visible The woman died, unfortunately, from a profuse intra-peritoneal hemorrhage as a result of the liver biopsy and at the autopsy performed shortly afterwards the histologic picture of the liver was entirely changed A dissruption of the so-called liver cell cords (or better said, plates, according to Elias(2)) was noted in that the individual cells were now apart

* Supported by a grant from the Dr Jerome D Solomon Memorial Research Foundation

trarily graded without reference or knowledge of the diagnosis by at least two independent observers A given phenomenon was statistically correlated by Franklin and co-workers(7), with abnormalties in various hepatic tests performed at the time of the biopsy. Obviously, such correlations do not mean causal relations but rather associations. This should reveal trends which may not be necessarily true in individual cases. However, such a statistical analysis can take the coincidence of anatomical and functional features out of the realm of impression based on observations of individual cases and make them generally valid. To review these findings rapidly (because many of you may possibly have seen Table II before (8)), the most significant statistical correlations were found in the case of diffuse liver cell damage. It correlated especially with abnormal results in cephalin flocculation, thymol turbidity, albumin/globulin ratio and bromsulphalein retention. In contrast to this, focal necrosis failed to reveal any significant correlation There were other relations such as that of regeneration with thymol turbidity or of distorted reconstruction of the lobular pattern (cirrhosis formation) with cephalin flocculation, thymol turbidity and sedimentation rate. Periportal inflammatory activity showed relation mainly to sedimentation rate, and Kupffer cell activity to the albumin/globulin ratio and to the serum bilirubin The presence of fatty metamorphosis failed to reveal any correlation Since the presence or absence of liver cell damage showed the best correlation, we became interested in finding out whether a quantitative correlation exists between the degree of liver cell damage and the degree of abnormality of the hepatic tests

These studies provide also the opportunity to recognize the characteristics of different degrees of liver cell damage in hematoxylin-eosin specimens, without the use of special technique

Steigmann, Szanto and I(9) described four degrees of liver cell damage We considered as grade zero liver cell damage a picture found in only 7 of our specimens In those the appearance and staining of the liver cells was, in general, uniform The size of the nuclei varied in the different zones of the lobule, however, in the same zone they were of equal size In grade I liver cell damage moderate variation in the size of cells and nuclei, even in the same zone, was noted and the cytoplasm was rather uniform but the cell borders were poorly seen We felt that this grade I liver cell damage was still insignificant In grade II liver cell damage liver cells revealed, in addition to moderate irregul...

TABLE II
STATISTICAL RELATION BETWEEN RESULTS OF HEPATIC TESTS AND PATHOLOGICAL PHENOMENA WITHOUT REFERENCE TO DIAGNOSIS

	Diffuse liver cell damage	Focal Necrosis	Regeneration	Distorted Reconstruction	Periportal inflammatory activity	Fatty metamorphosis	Kupffer cell activity
Cephalin cholesterol flocculation	+++	0	0	+++	+	0	0
Thymol turbidity	+++	0	++	++	0	0	0
Total serum protein	0	0	0	0	0	0	0
A/G ratio	+++	0	0	0	0	0	+++
N P.N.	0	0	0	0	0	0	0
Urinary urobilinogen	0	0	0	0	0	0	0
Stool urobilinogen	0	0	0	0	0	0	0
Total cholesterol	0	0	0	0	0	0	0
Cholesterol ester	0	0	0	0	0	0	0
Serum bilirubin	+	0	0	0	0	0	0
Bromsulphalein	+++	0	0	0	0	0	+++
Alkaline phosphatase	0	0	0	0	0	0	0
Prothrombin time	+	0	0	0	0	0	0
Vitamin A	+	0	0	0	0	0	0
Sedimentation rate	0	0	0	++	++	0	0

Reprinted from article by H. Popper *et al* Am J Med 6, 278 (1949)

trarily graded without reference or knowledge of the diagnosis by at least two independent observers A given phenomenon was statistically correlated by Franklin and co-workers(7), with abnormalties in various hepatic tests performed at the time of the biopsy. Obviously, such correlations do not mean causal relations but rather associations This should reveal trends which may not be necessarily true in individual cases. However, such a statistical analysis can take the coincidence of anatomical and functional features out of the realm of impression based on observations of individual cases and make them generally valid. To review these findings rapidly (because many of you may possibly have seen Table II before (8)), the most significant statistical correlations were found in the case of diffuse liver cell damage. It correlated especially with abnormal results in cephalin flocculation, thymol turbidity, albumin/globulin ratio and bromsulphalein retention. In contrast to this, focal necrosis failed to reveal any significant correlation There were other relations such as that of regeneration with thymol turbidity or of distorted reconstruction of the lobular pattern (cirrhosis formation) with cephalin flocculation, thymol turbidity and sedimentation rate. Periportal inflammatory activity showed relation mainly to sedimentation rate, and Kupffer cell activity to the albumin/globulin ratio and to the serum bilirubin. The presence of fatty metamorphosis failed to reveal any correlation. Since the presence or absence of liver cell damage showed the best correlation, we became interested in finding out whether a quantitative correlation exists between the degree of liver cell damage and the degree of abnormality of the hepatic tests.

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marked variations in size and either nuclear alterations or cytoplasmatic changes of moderate degree which may include marked vacuolization or the appearance of strongly basophilic or even more significantly, eosinophilic areas of smudgy appearance which represent coagulation necrosis. In grade III these changes were more pronounced (Figure 1). The appearance may vary markedly from cell to cell. Some cells revealed hydropic swelling, were rather large and appeared empty, changes which have been associated by Gillman and Gillman(10) with anoxia. In other ballooned cells basophilic material was clumped. Others presented eosinophilic clumped areas, some were frankly necrotic without nuclei and some were fragmented.

Two statistical methods were applied to determine the correlation between the degree of liver cell damage and the degree of abnormality, namely, the T test for the significance of difference between 0 and 1+ liver cell damage and 2 and 3+ liver cell damage and, in addition, the Chi square. With both methods a good correlation was found with an increase in cephalin flocculation and thymol turbidity and a decrease in the albumin/globulin ratio, whereas the globulin elevation was just on the border of significance. Total cholesterol, prothrombin time, alkaline phosphatase, urinary urobilinogen excretion and sedimentation rate failed to show correlation (Table III).

An attempt was made to study the correlation of liver cell damage with cephalin flocculation, thymol turbidity and alkaline phosphatase in individual diseases (Figure 2). In infectious hepatitis the correlation with cephalin flocculation is very good. In toxic hepatitis it only appears with severe liver cell damage. In extrahepatic biliary obstruction without infection (biliary hepatitis) the cephalin flocculation may be negative even with severe liver cell damage whereas with infection (purulent hepatitis) a correlation does appear. With thymol turbidity the correlation is not as clear, though of similar nature. The level of the alkaline phosphatase, however, appears far more related to the obstructive phenomenon than to the liver cell damage as can be recognized from the high levels occurring in both biliary and purulent hepatitis independent of the degree of liver cell damage.

Similarly, Waldstein, Szanto, Steigmann and Popper(11, 12) correlated statistically the histologic findings in 117 biopsy specimens of hepatic cirrhosis with various clinical and laboratory find-

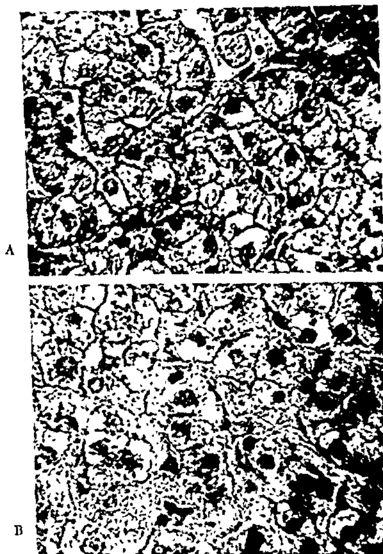


FIGURE 1

Photomicrographs of human liver stained with hematoxylineosin (450 x).

A) Zero degree liver cell damage. The liver cells and their nuclei are of equal size, their cytoplasm appears finely vacuolated and granulated due to the presence of [illegible]

B) [illegible]

TABLE III

STATISTICAL CORRELATION OF THE RESULTS OF HEPATIC TESTS WITH THE DEGREE OF HISTOLOGIC LIVER CELL DAMAGE INDEPENDENT OF DIAGNOSIS

TEST	T test for significance of difference of + from ++ or more liver cell damage (significant above 2.5)	Chi square to measure association of changes (significant above 16.8)
Cephalin flocculation	5.3	36.1
Thymol turbidity	4.1	26.2
Total protein	0 3	12 9
Albumin	3 6	21.2
Globulin	2.5	14 0
Albumin/globulin	3.7	16 4
Total cholesterol	1.5	5 6
Prothrombin time	0.1	4.4
Alkaline phosphatase	0 9	15 5
Urinary urobilinogen	1.2	7.0
Sedimentation rate	0.1	2.0

ings. No attempt was made to differentiate Laennec's from postnecrotic cirrhosis, however, no samples of cardiac or biliary cirrhosis were included. In no instance did we find zero degree of liver cell damage, 10 cases revealed 1+, 65 cases 2+ and 42 cases 3+ liver cell damage. Some correlation of the degree of liver cell damage was noted with jaundice (total bilirubin levels above 2 mg per 100 cc.) and especially with marked degree of jaundice (total bilirubin levels more than 8 mg. per 100 cc. (Figure 3)). Almost 80 percent of the instances with 3+ liver damage had jaundice but icterus was present in only about 25 percent of those with 1+ liver cell damage. In no case with 1+ liver cell damage was severe jaundice noted A good correlation was noted with the laboratory findings at the time of the liver biopsy taken as a unit and termed "biochemical activity" as well as with clinical activity. The latter term is obviously vague and dependent on the subjective opinion of the clinician. Alcoholic history revealed a negative correlation in that all cases of our series with 1+ liver cell damage had an alcoholic history, whereas about 25 percent of the cases with 3+ liver cell damage had no alcoholic history. This means that in our material cases of cirrhosis without alcoholic history reveal more commonly severe liver cell damage than cases with alcoholic history.

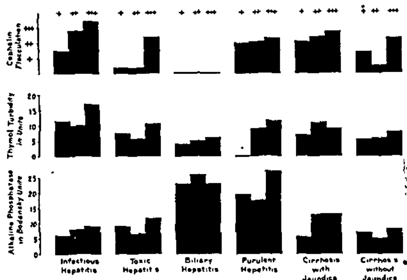


FIGURE 2

Average of results of hepatic tests (as indicated by the height of the column) in groups of patients with various degrees of liver cell damage in different hepatobiliary diseases Reprinted from article by H Popper *et al* *Am J Clin Path* 19, 710 (1949)

The attempt was further made to correlate the presence or absence of various other histologic features with the clinical findings (Table IV) Fatty metamorphosis showed correlation with alcoholic history and some with jaundice of moderate but not of severe degree. Ascites failed to reveal any correlation with liver cell damage itself and other phenomena related to it such as coagulation necrosis [illegible]

in the portal triads and with reconstruction of the lobular pattern as well as with angiogenesis which, according to Moschcowitz(13), probably represents anastomoses between the portal and hepatic circulation In short, we feel that the statistical analysis indicates what we would expect, namely, that features related to inflammation and scarring in the trabeculae are found more often than not in cases associated with ascites We have used the term "periportal" to indicate changes which take place in the trabeculae as well as

Watson: I would have thought it would be about two-thirds. That is what I figured from bedside observation.

Popper: The association of jaundice and ascites in the presence of cirrhosis was not as common in the material studied as in the data to which Dr. Watson and Dr. Handler refer. We deliberately took the presence of ascites or jaundice at the time when the biopsy was performed. In 23 percent of our cases jaundice and ascites were both present; in 28 percent both were absent; in 32 percent jaundice was present alone and in 17 percent ascites alone. That means that only in roughly 50 percent of the cases jaundice and ascites were simultaneously absent or present. The figures are a little different when the coincidence of jaundice and ascites, not at the time of biopsy but during the entire hospital stay, are considered. In this case both jaundice and ascites were recorded in 37 percent of our material and neither in 11 percent.

György: If you put jaundice and ascites together would you not get three plus correlation? You have to use several degrees of freedom. We are interested in the combination of ascites and jaundice together.

Popper. I suppose that if two degrees of freedom are used the correlation you mentioned would be found To continue, jaundice failed to reveal statistical correlation with any other of the listed phenomena. At this point we were interested in finding out whether the cases of cirrhosis with marked jaundice showed a characteristic histologic picture which would differentiate them from other cases without severe jaundice. We were especially interested in the type of cirrhosis in which an extremely high serum bilirubin or markedly elevated alkaline phosphatase was present or in which the urine and stool contained little or no urobilinogen. In those cases one may be justified to assume some interruption or disturbance of the bile flow and the name "cholestatic cirrhosis" has been applied. Obviously the question arises whether the histologic picture would possibly permit the recognition of the cause of the jaundice. Histologically in such cases very marked dilatation of the intralobular bile capillaries occurs which are filled with bile plugs. However, in the portal triads or trabeculae the bile ducts were free of bile pigment. In some instances the smallest bile ducts, the cholangioles, still contained bile pigment whereas little is found in the larger bile ducts This indicates that in this type of cirrhosis any obstruction or any other disturbance of the bile flow must be localized to the border zone between the lobular parenchyma and the portal space.

nisely: What is known about the solubility of bile pigments in histological fixing agents and the solutions of alcohol, xylol, etc., ough which the tissues have been run? Might the bile pigment absent because it would be dissolved out rather than because vould not have been present?

Popper: I cannot exclude the possibility that bile pigment be solved out from either location However, in extrahepatic me-nical biliary obstruction large amounts of bile pigment can be nonstrated in the bile ducts in the portal spaces which is not the e in the observed instances of so-called cholostatic cirrhosis. I not want to imply the presence of a mechanical obstruction at border of parenchyma and portal space, I just want to emphasize t a disturbance of the bile flow in this location is indicated by histologic evidence that bile pigment is found in the bile pas-ges up to and not beyond this point.

Knisely: One of the problems in all histopathology is as follows: e observes some material or object sitting in a vessel, and then erwards, says this material or object would obstruct the vessel wever, in the histological section there was no evidence by which know whether the object was sitting still or moving, either before after death The thing that triggered me was the word "obstruct." hen you said that, I thought you meant that the material was nding still and obstructing the vessel

Popper: Originally we felt(16) that this disturbance of the bile w is the result of an actual intrahepatic mechanical obstruction the smallest bile duct produced by a circular fibrosis. The latter a common picture in cirrhosis. However, we found a similar type d degree of fibrosis in many biopsy specimens of patients with rhosis who did not have a significant degree of jaundice or no undice at all (Figure 4) No correlation could be found between ch circular fibrosis and the degree of jaundice. In short, except r bile stasis there was no significant or regular difference in the stologic picture between cases of cirrhosis with severe jaundice nd those without. We have to conclude that our previous assump-on(16) about intrahepatic biliary obstruction was wrong.

Hanger: Was there bile in the stool of the patient whose biopsy as shown? Obviously there were also areas of uninvolved hepatic ssue excreting bilirubin If every bile duct had been like the ones own no bile could have been present in the stools.

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Popper: Originally we felt(16) that this disturbance of the bile flow is the result of an actual intrahepatic mechanical obstruction of the smallest bile duct produced by a circular fibrosis. The latter is a common picture in cirrhosis. However, we found a similar type and degree of fibrosis in many biopsy specimens of patients with cirrhosis who did not have a significant degree of jaundice or no jaundice at all (Figure 4). No correlation could be found between such circular fibrosis and the degree of jaundice. In short, except for bile stasis there was no significant or regular difference in the histologic picture between cases of cirrhosis with severe jaundice and those without. We have to conclude that our previous assumption(16) about intrahepatic biliary obstruction was wrong.

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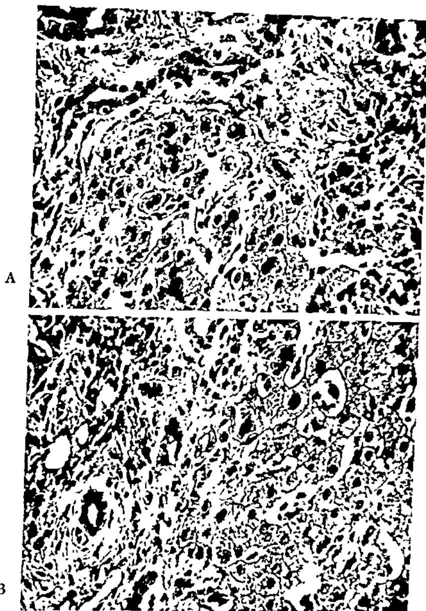


FIGURE 4

Photomicrographs of human livers stained with hematoxylineosin (230 x)

A) Cirrhosis with severe jaun [illegible] bile casts in dilated bile capillaries and cellular infiltration on the [illegible] nodule and in the portal space. The latter contains smallest bile [illegible] mar [illegible] ng is noted.

B) Cirrhosis without jaundice reve [illegible] the smallest bile ducts in the portal spaces [illegible]

Popper: In some cases with severe fibrosis in the portal triads, bile was found in the stool. Also in necropsy specimens of livers revealing cirrhosis without jaundice, severe portal fibrosis and similar scarring throughout the organ have been observed. This suggests that the lesion is not local and that such circular fibrosis does not suffice to obstruct the bile flow. At present we feel, therefore, that it is not an anatomically visible lesion that is responsible for this "obstructive feature" and for the severe jaundice in cirrhosis, but that the disturbance of the bile flow is rather, as Dr. Watson and Dr. Hoffbauer(17) have pointed out, the result of a permeability change of the bile ducts which permits the back flow of bile into the blood stream.

To continue, an attempt was made to correlate statistically ·t· presence or absence of various histologic phenomena with biochemical and clinical activity (Table V) In supplementati of the previously shown good statistical correlation between liv cell damage and these activities, inflammatory changes such a increased cellularity of the portal triads and the trabeculae, leuco cytic reaction especially in the portal areas, and small focal necroses also showed significant correlation, whereas the other features related to scarring or fatty metamorphosis fail to show any cor-

TABLE V

STATISTICAL SIGNIFICANCE OF CORRELATION BETWEEN CLINICAL AND HISTOPATHOLOGIC FEATURES IN CIRRHOSIS

	Biochemical Activity	Clinical Activity
Liver cell damage	+++	++
Coagulation necrosis	0	0
Fatty metamorphosis	0	0
Kupffer cell mobilization	0	0
Focal necrosis	+	+
Peripheral inflammation	0	0
Leucocytic reaction	+	+
Periportal cellularity	++	++
Fibroblastic proliferation	0	0
Fibrocytic proliferation	0	0
Periportal scarring	0	0
Altered reconstruction	0	0
Bile duct proliferation	0	0
Angiogenesis	0	0

relation. We find thus an overlap in the statistical analysis in that both liver cell damage and inflammatory features are associated with biochemical and clinical activity. The question arose which of the two is actually related to the activities in cirrhosis. In 18.6 percent of the clinically active and in 18.4 percent of the biochemically active cases liver cell damage was noted without any inflammatory features. In 2.3 percent of the clinically and in 1.1 percent of the biochemically active cases there were inflammatory features (none with regularity) observed without liver cell damage. These figures indicate that liver cell damage is the histologic feature associated regularly with clinical and biochemical activity in hepatic cirrhosis. Inflammatory changes, which are so commonly seen in the presence of liver cell damage accompany the latter and are possibly caused by the latter rather than represent the cause for this activity.

Kinsell: By "inflammatory change" you mean round cell infiltration?

Popper: Under inflammatory changes, we include infiltration by round cells of histiocytic and lymphocytic character as well as by segmented leucocytes.

Hanger: I agree with that, except for one thing: Liver cell damage is too general a word I think the more the necrosis factor is dominant, the more severe are the associated inflammatory changes. Certain types of injury of liver cells might be compared to the withering seen in a sickening plant. Death of the structure may be so gradual that secondary reactions in the contiguous tissues do not take place. Fatty infiltration of parenchymal cells and certain of the changes which you mentioned permit the injured cell to remain viable, and hence inflammatory changes in the mesenchymal structures are not invoked. The cells must disintegrate to cause positive cephalin flocculation reactions. Some types of injury leave the nonviable cells *in situ*, causing a hyalin or fatty transformation rather than autolysis I think the derivatives of cellular breakdown are important factors in the production of inflammatory reactions in the liver

Popper: I agree with you However, I would like to qualify your statement with one more thought In certain forms of necrosis or necrobiosis, as in viral hepatitis, the mesenchymal reaction is extremely marked The question arises whether this mesenchymal inflammatory reaction is directly provoked by the virus or whether it is a response to the liver cell damage. In contrast, in the hepatic

necrosis produced by chemicals in the human, the inflammatory reaction is, as a rule, less marked, is often missing and if present consists more of segmented leucocytes than of round cells which predominate in the viral hepatitis. We feel thus that not only the presence but also the type of necrosis decides the appearance of the inflammatory reaction

Also in cirrhosis the attempt was made to correlate liver cell damage with the results of the various hepatic tests. Again, as in the entire series so far, the best correlation was found with elevated cephalin flocculation and thymol turbidity; slightly less so with reduced serum albumin and elevated serum globulin and consequently also with reduced albumin/globulin ratio. R[illegible] cholesterol ester percentage re[illegible] the serum total choleste[illegible] tion and sedimentation r[illegible] [illegible]ical significant correlation. [illegible] There-

Patek I would infer that the statistical correlation was made with heterogeneous groups, or several types of liver damage Do you feel that the correlation would be equally valid for homogeneous groups as well?

Popper The greatest difficulty in any such study will be the size of the sample Since we have so much overlap of the different histologic features, a large sample is required to come to a statistically valid conclusion Therefore, for the time being, heterogenous groups are taken with the hope to get some type of correlation We are sure that the data presented are not a definitive type of study To do the latter will only be possible if the statistical sample will entail a thousand or more specimens Then it will be possible to divide it into different disease groups and homogenous sub-groups We hope, thus as time goes on, to have sufficient material, for instance to use the same approach in viral hepatitis

Kinsell How do you evaluate Kupffer cell activity? I am asking for information because we have not been able to do this to our satisfaction

Popper As far as Kupffer cell activity is concerned, we determined presence and absence and not its degree If more than half of the sinusoidal endothelial cells are bulging forward into the lumen or appear almost completely detached and if their number is increased we consider Kupffer cell activity as present

Kinsell May I comment on the Kupffer cell activity? A phagocyte can ingest and digest something like a red cell rapidly We have

of our histologic findings shown here are subject to criticism from a normal histologic viewpoint. However, that is the material we are forced to work with. Those here who have seen cases of viral hepatitis will surely agree that the Kupffer cells in this condition are large and swollen. What this means is subject to speculation.

Fremont-Smith: This illustrates the value, does it not, of specifying your basic assumption? I believe that one can say here is a change. Then one may or may not go forward with the interpretation of that change in terms of function, and Dr Knisely is pointing out the danger of the interpretation in terms of function when you don't have a sequential picture which is related to the time sequence of the process.

Best: It seems to me, Dr. Popper, that you say the routine procedures which we have to use are full of fallacies. Why do we have to go on using them? They do not have to be the routine.

Popper: Vital microscopic examination of the liver may give some additional information, but for the time being the pathologists have to try to learn as much as possible from the small needle core obtained by liver biopsy. The presented study tries to determine the information obtainable with the classical method of histology and to check impressions of coincidences of various features by statistical analysis. I fully agree with your criticism that if we see an enlarged Kupffer cell in a fixed tissue slide we may have no idea of what this enlargement means, but we want to know whether and how regularly this enlargement occurs in certain conditions or diseases The presented study of statistical coincidences, not of causative relations, taught us two things: a) as far as liver cell damage is concerned the small biopsy specimen is representative whereas in most other features it may not always be, b) liver cell damage reveals the best correlation with clinical activity as well as with the results of the hepatic tests.

We were thus interested in a better method for the recognition of liver cell damage than the routine hematoxylineosin stain. Szanto, in our laboratory (19), therefore, studied the so-called basophilic bodies in the cytoplasm Many substances in the tissues with acid valences are removed during fixation and preparation of the tissue [illegible] are the acid mucopolysac- [illegible] their phosphoric [illegible] cur in liver cells. acid radical. Since the former [illegible] most of their basophilia is, therefore, due to nucleic acids. These

are found in the nucleus as desoxypentose nucleic acids which combined with some proteins, mostly of histone character, form the chromatin. In the nucleolus and cytoplasm they are present as pentose nucleic acids, again, at least in the liver, bound to protein moieties. The basophilia can be recognized in the routine hematoxylin stain but is seen better with either gallocyanin or Giemsa stains and especially the methylgreenpyronin stain in which desoxypentose nucleic acid stains green and the nucleoli and the cytoplasmic basophilic material of pentose nucleic acid nature stains red. The evidence that all this basophilic or pyroninophilic material is actually pentose nucleic acid is far from being complete, that the greater part of it is, has been demonstrated by various techniques. Caspersson(20), Pollister and Ris(21) and others demonstrated by ultraviolet microscopy that this material absorbs specifically the same ultraviolet rays which nucleic acid absorb. Brachet(22) and others digested the basophilic material with the specific enzyme ribonuclease and the same technique for identifying this material was used in our studies. The entire basophilic material of the liver is removed by this specific digestion. The validity of this evidence, however, that the material is pentose nucleic acid depends upon the specificity of the enzyme used and upon its freedom from other proteolytic enzymes. In other organs like the thyroid, acid proteins besides ribonucleic acid compounds have been demonstrated by Pollister and Leuchtenberger(23) but at present it appears acceptable that in the liver at least the staining with pyronin and the other dyes mentioned is specific for pentose nucleic acid especially if controlled by specific digestion with ribonuclease.

What is the function of the pentosenucleic acid in the cells? Caspersson(20) and Brachet(22) have brought forward evidence that pentose nucleic acid plays an important part in cytoplasmic protein formation. This evidence is partially based on the fact that it is found in high concentration in cells which are known to be very active in protein synthesis. These are the pancreatic cells, hepatic epithelial cells, nerve cells, basophilic cells of the anterior lobe of the pituitary gland, lymphocytes and plasma cells, as well as embryonic and cancer cells. In addition there are a series of physiologic observations supporting this hypothesis which were recently summarized by Brachet(24) and which I would not like to repeat in detail. A close correlation between protein formation and the pentose nucleic acid of the liver has been demonstrated by Lagerstedt(25) and here and in other organs it has been assumed

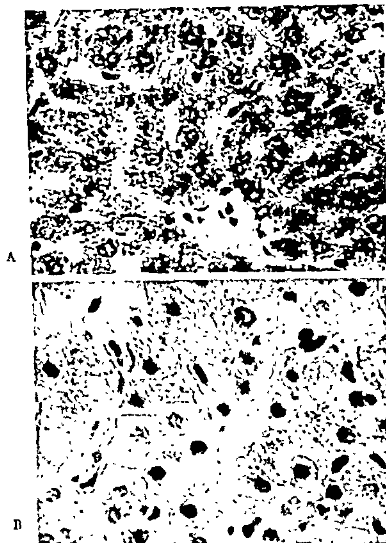


FIGURE 5

Photomicrographs of human livers stained with methylgreenpyronin (450 x)

A) Normal liver. The cytoplasm of the epithelial cells contains large amounts of basophilic granules, the cytoplasm of the mesenchymal cells is free of it

B) Nodule in active cirrhosis: the cytoplasm of the epithelial cells is free of basophilia

that the nucleolar pentose nucleic acid is under the influence of the nucleolus-associated chromatin and in turn, regulates the cytoplasmic pentose nucleic acid. Nevertheless, the evidence concerning the relationship between protein synthesis and pentose nucleic acid is, so far, only circumstantial and the biochemical nature of the link between pentose nucleic acid and protein synthesis is not clear. To date, it cannot be excluded that pentose nucleic acid is present in these locations as the result rather than the cause of protein formation. However, it may be permissible without reference to this argument to consider the presence of pentose nucleic acid as a sign post of protein synthesis with all reservations necessary in histochemistry.

The cytoplasmic basophilic material mostly appears granular in the tissue section and Opie(26) assumed a close relation between it and mitochondria. In ultracentrifugation studies this material is mainly found in the granular (mitochondrial and microsomal) fraction and little in the supernatant fluid. How then is the distribution of these basophilic bodies altered in diseases of the liver? They appear denser in brown atrophy or in various forms of compression atrophy; the increase in basophilia is more apparent than real since the basophilic bodies are concentrated in a small cell volume. Pigmentation in moderate amount does not influence the basophilic bodies in the liver cells and bile pigment deposition is not always combined with decreased basophilia. Also fatty metamorphosis of moderate degree is not necessarily associated with reduction of the basophilia; however, in more advanced degrees the cytoplasmic basophilia is markedly reduced. In some types of cytoplasmic dis- [illegible] forms of coagula- [illegible] ire 5). [illegible] tholo- gist may differentiate an acidophilic (eosinophilic) degeneration if the nuclei are still intact and an *acidophilic necrosis* if marked nuclear changes are noticed [illegible]

ba [illegible] from the cytoplasm of the cells [illegible] normal circumstances the basophilic cytoplasmic bodies indicate the amount of pentose nucleic acid present. Whether this holds true under pathologic circumstances required chemical analysis. Therefore, Farber, Koch-Weser and Szanto(27) compared, in our laboratory, the degree of visible basophilia in liver sections of fasted

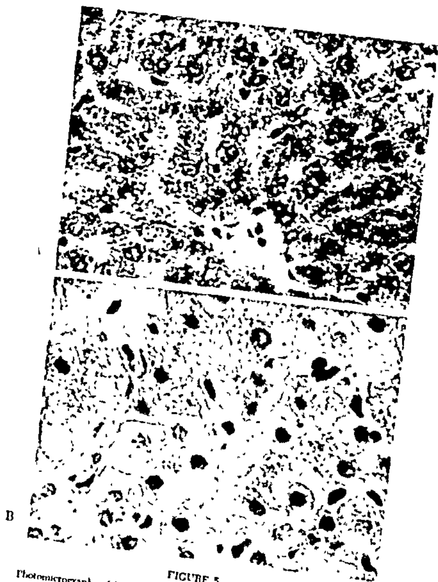


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A: Normal liver. The cytoplasm of the epithelial cells contains large amounts of basophilic granules; the cytoplasm of the mesenchymal cells is free of it.

B: Nodule in active cirrhosis; the cytoplasm of the epithelial cells is free of basophilia.

female rats intoxicated with carbon tetrachloride and ethionine, with quantitative chemical determinations of hepatic concentration and amount of total, pentose, and desoxypentose nucleic acids. In carbon tetrachloride intoxicated rats there was a loss of basophilia in the necrotic centro-lobular and fatty intermediate zones. After ethionine administration a diffuse fatty metamorphosis developed in combination with a marked, diffuse decrease of basophilia in the liver. At 24 hours after the intoxication these changes were associated with a decreased concentration of the total as well as of the pentose nucleic acid, if they were expressed in mg. per 100 grams liver. However, this drop was not as marked as would appear from the reduction of the histologically visible basophilia (Figure 6). After 48 hours, in carbon tetrachloride intoxicated rats the nucleic acid concentration increased probably due to regeneration in the peripheral zone. After 120 hours the concentration of the nucleic acid as well as the distribution of the basophilia was, in all instances, practically identical with that of the fasted controls. The total nitrogen of the liver decreased in all animals, the intoxicated as well as the controls, due to the starvation.

If the nucleic acid values were expressed not as concentration (in mg. per 100 grams liver) but in total amount (mg. per 100 grams initial body weight) the reduction observed in concentration was not noted and the total nucleic acid amount remained constant. It even rose subsequently in carbon tetrachloride intoxicated rats due to the regeneration This is explained by the fact that in the intoxicated rats the liver as a whole becomes larger and the actual loss of pentose nucleic acid if present at all, must be very small. The reduction of histologically recognizable basophilia may be partially explained by a mere dilution of the basophilic material due to the swelling of the cells However, it cannot be excluded that in the fatty, necrotic and even prenecrotic cell the physicochemical state of the pentose nucleic acids (possibly their protein combination) is changed so that they do not exhibit basophilia, but are still demonstrable by chemical methods In this sense, the basophilia may be a better index of the functional integrity of the cell than the chemical analysis; on the other hand, these investigations point out how dangerous it is to draw conclusions about the pentose nucleic acid content of the liver from a microscopic examination of sections from abnormal livers

The basophilic bodies in the Kupffer cells also deserve some interest Under normal conditions hardly any bodies can be demon-

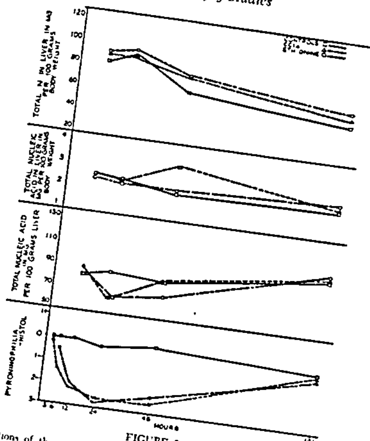


FIGURE 6

Variations of the amounts of total nitrogen and of total nucleic acids in liver (both expressed in mg per 100 Gm initial body weight), of the hepatic concentrations of total nucleic acids (expressed in mg per 100 Gm liver), and of the histologically graded degree of depletion of cytoplasmic basophilia of the liver cells in normal starving rats as well as in starving rats intoxicated with carbon tetrachloride (0.01 cc per 100 Gm body weight given intraperitoneally) and with ethionine (0.1 Gm per 100 Gm body weight given intraperitoneally)

strated in their cytoplasm However, in viral hepatitis and to a lesser degree in cirrhosis, the large, bulging Kupffer cells contain many basophilic bodies Similarly, wandering or fixed histiocytic cells in the portal triads and trabeculae stand out by their basophilic cytoplasm especially in cirrhosis This represents a morphologic sign of the activity of these mesenchymal cells (Figure 7)

Watson How do you interpret these cells? Do you think that they are derived from the Kupffer cells or represent histiocytes?



FIGURE 7

Photomicrographs of human liver stained with methylgreenpyronin (450 x).

A) [illegible] a case of active cirrhosis The cytoplasm of the accumulated [illegible]

B) [illegible]

nonbasophilic cytoplasm contains many bile pigment [illegible]

Popper: These cells may be Kupffer cells or histiocytic cells, which may or may not have been formed in the portal or trabecular connective tissue.

Knisely: Recent studies on Kupffer cells show that the Kupffer cells migrate to that position (studies by Carl Mortenson of the Anatomy Department, University of Wisconsin). The evidence suggests that the Kupffer cells migrate to that region and retain that material.

Popper: However, not all enlarged or "active" hepatic mesenchymal cells reveal cytoplasmic basophilia. For instance, in extrahepatic biliary obstruction the enlarged Kupffer cells, which are loaded with bile pigment, fail to show this basophilia.

The study of the distribution of the basophilic bodies may also be of help in the histologic differential diagnosis of various hepatic diseases. For instance, in benign viral hepatitis the spotty character of the necrosis is emphasized in pyronin stains in that scattered irregularly throughout the lobules, one notes cells depleted of basophilia, whereas other hepatic cells stand out by their basophilic cytoplasm. The picture is made more characteristic by the marked basophilia of the Kupffer cells and portal mesenchymal cells. In contrast, in the toxic type of hepatitis, as for instance in carbon tetrachloride intoxication, the zonal depletion of the basophilia, mostly in the central zone, is characteristic, in the peripheral zone a high amount is noted, probably due to regeneration in accordance with what we have just seen in rats 48 hours after carbon tetrachloride intoxication. In the human, the mesenchymal reaction is less pronounced or completely absent. In cirrhosis the basophilia of the hepatic cells may offer a differentiation between the active and inactive form. Depletion of the hepato-cellular basophilia, usually associated with marked basophilia of the Kupffer cells and especially of histiocytes in trabeculae and portal triads, characterizes the clinical and biochemical active cases. There is great variation from lobule to lobule, but in the inactive form almost all lobules are rich in basophilic material.

Does this loss of basophilia of the hepatic cells in cirrhosis indicate impaired protein formation? We are still hesitant to draw this conclusion. The protein formation may not necessarily be decreased despite the absence of basophilia. As mentioned before, the nucleolus is supposed to control the cytoplasmic nucleic acids. In cirrhotic livers large nucleoli may occasionally be seen and although

the cytoplasm may appear rather empty, basophilic material may accumulate as a small rim around the nucleus. The picture resembles that observed by Lagerstedt(25) in protein-depleted animals which receive large amounts of protein. The histologic picture does not exclude the possibility that in the cirrhotic liver a marked turnover of pentose nucleic acids takes place with rapid formation in the nucleolus and the perinuclear zone. It would, therefore, be in accordance with very rapid formation and destruction of protein as well as with decreased formation. We feel that the study of the basophilic material may assist us in the histologic recognition of liver cell damage and also in the appreciation of some functional changes, especially as far as protein synthesis is concerned which represents one of the main functions of the liver.

Dr. Szanto tried to correlate in different conditions the serum albumin concentration at the time of the liver biopsy with the depletion of the basophilia Although no statistical correlation could be demonstrated he found a tendency for the serum albumin to be low in the instances of marked depletion. This holds true for cirrhosis especially. We do not consider this observation as proof that albumin formation is directly related to the hepatic pentose nucleic acid because liver cell damage may otherwise be related to low serum albumin and run parallel with reduction of the basophilia.

There is fairly good evidence that serum gamma globulin is formed by mesenchymal elements and in the liver this would implicate the Kupffer cells and the mesenchymal cells in the portal triads Actually, the serum gamma globulin level as determined by electrophoresis, or turbidimetrically, is usually high in diseases in which there is marked mobilization and activity of the Kupffer cells and other hepatic mesenchymal cells and in which their cytoplasm is basophilic(28, 19) This holds true for infections and less so for toxic hepatitis and is especially true in cirrhosis, in which the serum gamma globulin level is high (Figure 8). In contrast, in obstructive jaundice in which the bile-laden Kupffer cells, though histologically active, are free of basophilia, the serum gamma globulin level is usually slightly, if at all, elevated Obviously, in the individual case the correlation between the histologic grading of the basophilic reaction of the mesenchymal cells and the serum gamma globulin level is not always present. However, we assume here a possible chance to read into the histologic findings some functional meaning and some support for the theory that the ele-

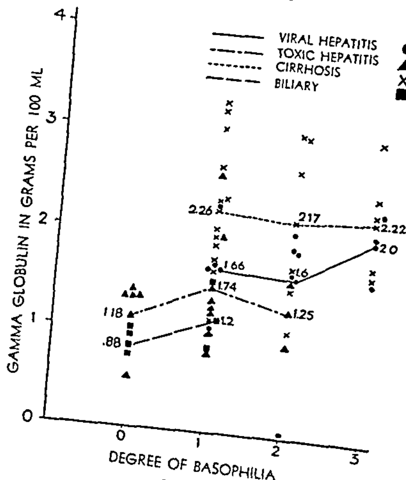


FIGURE 8

Correlation of the histologically graded amount of cytoplasmic basophilia of the hepatic mesenchymal cells with the serum gamma globulin concentration determined at the time of the biopsy in various hepato-biliary diseases

...ated gamma globulin level in hepatic diseases is related to the demonstrated mesenchymal reaction

Hanger Do you find any differences between the early stages of ...xic hepatitis and long-standing involvement? For instance, it is ...sual to observe certain protein changes, i.e., positive cephalin ...cculation twenty-four hours after an acute parenchymal damage. ... positive thymol turbidity reaction may be absent or may not

develop for some time, presumably after the development of mesenchymal irritation. There is very little difference between your infectious and your toxic groups, but I would suspect that during the early stages you would not find elevated gamma globulin in toxic hepatitis.

Popper: I do not think we have any early cases.

Best: Are there any further questions on this presentation?

Gordon: Dr. Popper's data bring up a very troublesome point which is fundamental in any quantitative enzyme work on tissues. I am sure that Dr. Stetten and others will bear me out on this, namely, the question of changes in concentration of enzymes in relation to a base of reference. For example, we have been doing a great deal of quantitative enzyme work in liver damage using radiomethionine as a tool, and we find a fall, for instance, in succinic dehydrogenase. Is it really a fall or is it due to the fact that there is increase in hydration of liver cells or increase in liver fat? In other words, does it represent a real decrease in enzyme concentration? We attempted to get around that by determining on the basis of wet weight, dry weight, total nitrogen, fat free nitrogen, to eliminate the phospholipids, and we finally came around to the conclusion that ribonucleic acid was possibly the best basis of reference upon which to judge enzyme concentrations

Dr. Van Potter, a very excellent enzyme chemist working at the University of Wisconsin, whose judgment I value very much, simply reports his data on the basis of wet and dry weight, feeling that the magnitude of changes that he is looking for is great enough so these changes will be reflected anyway. But this is a very troublesome point, and I wonder whether anyone else has any solution to this problem as to what to use as a basis for reference in enzyme concentration

Handler: Dr. Earl P. Benditt of Chicago, at the Rutgers' Protein Symposium last January, indicated the variability of liver ribonucleic acid and showed a correlation between the decreased ribonucleic acid and the disappearance of mitochondria in protein depleted rats As was, perhaps, to be expected, this paralleled the diminution in activity of several enzymes, notably succinoxidase. But the desoxyribonucleic fraction did not seem to diminish particularly and it might serve as the desired standard.

Gordon: Ribonucleic acid phosphorus using Schneider's technique could be used as a basis of reference.

Tarver. The other approach is to make comparisons of total enzyme per unit of intact animal, or relative to an intact animal under some predetermined standard conditions.

Stetten: I think this matter of primary standard has been threshed out in the presence of many of the gentlemen in the Sub-Committee of Liver Disease of the National Research Council. Desoxypentose-nucleic acid is a very appealing substance for another reason, namely, on the basis of purine turnover it is one of the few compounds that apparently does not turn over much at all in tissues in which mitosis is occurring. So at least in liver disease where there is no major regeneration one might anticipate the concentration of desoxyribonucleic acid should be a relatively constant quantity.

Watson: What about the specific gravity of the liver in terms of simply measuring specific gravity of biopsies? It does not seem that anyone has ever done that. I wondered whether it would not be a good thing to do routinely, and if we would not get more information by taking a little bit of our biopsy and determining the specific gravity of it. After all, the two main elements we are worried about are water and fat. Would we get the information? Perhaps the simplest and best standard would be if we measured the specific gravity.

Knisely: The methods of measuring the specific gravities of drops of blood with solutions of different density might be applied to this problem.

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Kunsely: The methods of measuring the specific gravities of drops of blood with solutions of different density might be applied to this problem.

was negative and the thymol turbidity and serum alkaline phosphatase were normal In spite of the slight discrepancy in the laboratory tests, we thought that we were dealing with some form of hepatitis and were quite amazed to find metastatic ovarian carcinoma in the biopsy specimen.

Another example was the case of a colored man of fifty-five, who unfortunately had his eyes enucleated, so it was impossible to know how long he had had jaundice. As a matter of fact, the icterus was discovered as a result of a lumbar puncture done for follow-up study. He had received arsenical therapy five or six months before. There were anorexia and diarrhea His liver was enlarged and the spleen was palpable. There was a severe degree of jaundice and the cephalin flocculation test was three plus, the thymol normal and the serum alkaline phosphatase somewhat increased In view of the fact that he had received arsenic, and in view of the physical findings, we thought he had homologous serum hepatitis or a possible arsenical hepatitis, if you wish. The biopsy specimen showed the findings characteristic of obstructive jaundice including a bile lake (Figure 9). When we found the bile lake, we advised immediate surgery. At operation the spleen and liver were enlarged. There was no distension of the gallbladder or common duct, and no tumor was palpable in the region of the ampulla of Vater or head of the pancreas. The surgeon said, "I think your original clinical diagnosis of hepatitis is correct." I said, "You had better open the common hepatic duct and make sure," which he did, and found a carcinoma of the main hepatic duct. It was the presence of this bile lake that made us lean so strongly toward extrahepatic obstructive jaundice

A third example is that of a woman of thirty-three, who had pain in the right upper abdominal quadrant with nausea and vomiting five weeks before admission. She was jaundiced for one week and comatose for one day We could not feel the liver. She had an icteric index of 125, and a positive cephalin flocculation test. We could not explain the coma on a hypoglycemic or other basis. The presence of jaundice and coma led us to believe that we were dealing with acute yellow atrophy.

On liver biopsy, we were surprised to find the changes of obstructive cirrhosis with very little evidence of hepatic cell injury except for scattered areas of focal necrosis (Figure 10). There was certainly quite a discrepancy between the biopsy findings and the clinical diagnosis Whether the coma was of hepatic origin, we are not sure Ot least, there is very little in the histologic change in

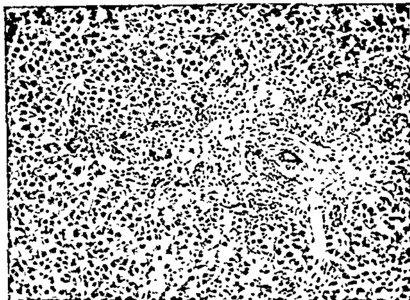


FIGURE 10—A F. (G E. 19)

Biliary Cirrhosis, Obstructive A portal area demonstrating fibrosis, bile duct proliferation and a moderate leukocytic infiltration Save for bile stasis, which is not well shown in the illustration, the parenchymal cells are unremarkable

the liver in keeping with such an assumption She was given supportive treatment, came out of her coma, and at laparotomy later on, a stone was removed from the common bile duct A liver biopsy eighteen months later still showed periportal fibrosis with otherwise normal liver parenchyma. We wanted to see whether the obstructive cirrhosis had disappeared, but it had not.

The following case illustrates a perplexing problem in the field of obstructive jaundice, viz, the status of intrahepatic obstructive jaundice The patient was a white male of forty-one, a severe diabetic, who became jaundiced on March 1, 1949. His liver was pal[illegible]
an[illegible]
neg[illegible]
alk[illegible]
month A liver biopsy showed bile stasis and some focal necrosis, and was interpreted as indicating obstructive jaundice. The liver profile was in keeping with such a diagnosis and, because he was

a diabetic, we thought he had a carcinoma of the head of the pancreas. At operation, no obstructive lesion was found. The gallbladder and common bile duct were not distended. Cholangiograms were negative. The surgeon did put in a "T" tube. There was very little bile flow, and it looked like we had an intrahepatic obstructive type of lesion.

He continued to be jaundiced, and about six months after the onset of jaundice, in September, 1949, we did another liver biopsy, with the same findings as in the initial specimen We watched him through thirteen months of jaundice. The cephalin flocculation remained persistently negative, the serum alkaline phosphatase persistently increased and the total blood cholesteral at a level of 1400 mg. percent. Eventually he developed xanthomata on his elbows and palms We did another biopsy at the end of about eleven and one-half months of jaundice There were still the same changes of bile stasis and focal necrosis. We showed the microscopic specimen to Dr. Watson, and he saw the patient in consultation. We also sent slides to Professor Dible in London.

The patient died after thirteen months of jaundice. Toward the end, his icterus became quite deep, with the total serum bilirubin over forty mg percent. He developed an abnormal mediastinal shadow and a shadow in the right lung hilum which were due to lymphoma. Post-mortem examination revealed the liver to show only bile stasis and focal necrosis. The lymphoma did not involve the liver or liver area.

Watson: There was no dilution of the extrahepatic ducts?

Schiff No No lymphoma was found in the region of the liver.

Hanger: And the portal triads looked perfectly normal?

Schiff. They showed very little, if anything, Dr Hanger.

Watson. Just a functional disease is what this patient had, a cholangiolitic type?

Schiff: Cholangiolitic hepatitis I presume, Dr. Watson, with thirteen months of jaundice and no progressive histological changes in the liver.

A man of sixty-four came in when he was jaundiced about two weeks, rather deeply jaundiced, with a four plus cephalin flocculation, increased thymol turbidity and a serum alkaline phosphatase of five Bodansky units. We could not get a good history from him as he had been in a mental institution. With a profile such as he

presented, we thought he had hepatitis. A liver biopsy showed the presence of cholangitis with some focal necrosis, not very much, and the pathologist thought that the changes were associated with an obstructive lesion. He became much more deeply jaundiced in short order, the serum bilirubin rising to 51 mg. percent He developed an hepatorenal syndrome with nitrogenous retention, oliguria, etc Even though we now suspected extrahepatic involvement, the hepatitis element was so prominent we did not dare to interfere surgically. The predominant clinical picture was that of a severe hepatitis, which apparently was secondary to extrahepatic disease, namely, common duct stone, which was subsequently removed The first suspicion of extrahepatic disease was revealed by liver biopsy

Liver biopsies are of value in following the course of liver disease They are the best means of making sure that a hepatitis has completely disappeared and of following the development of chronic hepatitis or cirrhosis

The biopsy of a man with homologous serum hepatitis, on the sixteenth day of jaundice, showed the presence of balloon cells, binucleate cells and intralobular infiltration. On the thirty-ninth day of jaundice he presented considerable fibrosis interpreted as a possible postnecrotic cirrhosis. Did we miss such a lesion on the previous biopsy? We do not know. We believe that the transition of hepatitis into cirrhosis may occur in rather short order, at least as based on biopsy studies although some may dispute the changes as indicating fibrosis rather than cirrhosis Such a rapid transition has been reported by Krarup(35).

A biopsy (Figure 11) was taken from a patient with sarcoidosis. He had scattered granulomata, which, though not specific for sarcoidosis, fit in well with the clinical picture ...

cirrhosis is present The relationship between granulomatous disease and cirrhosis has been hinted at in the literature, at least in the case of brucellosis(36, 37).

Liver biopsy is helpful in the evaluation of therapy in liver disease(38)

The liver biopsy from a case of fatty infiltration of the liver in a very fat woman, nonalcoholic, who ingests too little protein is

a diabetic, we thought he had a carcinoma of the head of the pancreas. At operation, no obstructive lesion was found. The gall-bladder and common bile duct were not distended. Cholangiograms were negative. The surgeon did put in a "T" tube. There was very little bile flow, and it looked like we had an intrahepatic obstructive type of lesion.

He continued to be jaundiced, and about six months after the onset of jaundice, in September, 1949, we did another liver biopsy, with the same findings as in the initial specimen We watched him through thirteen months of jaundice. The cephalin flocculation remained persistently negative, the serum alkaline phosphatase persistently increased and the total blood cholesterol at a level of 1400 mg. percent. Eventually he developed xanthomata on his elbows and palms. We did another biopsy at the end of about eleven and one-half months of jaundice. There were still the same changes of bile stasis and focal necrosis. We showed the microscopic specimen to Dr. Watson, and he saw the patient in consultation. We also sent slides to Professor Dible in London.

The patient died after thirteen months of jaundice. Toward the end, his icterus became quite deep, with the total serum bilirubin over forty mg. percent He developed an abnormal mediastinal shadow and a shadow in the right lung hilum which were due to lymphoma. Post-mortem examination revealed the liver to show only bile stasis and focal necrosis. The lymphoma did not involve the liver or liver area

Watson: There was no dilution of the extrahepatic ducts?

Schiff: No No lymphoma was found in the region of the liver.

Hanger: And the portal triads looked perfectly normal?

Schiff: They showed very little, if anything, Dr. Hanger.

Watson: Just a functional disease is what this patient had, a cholangiolitic type?

Schiff: Cholangiolitic hepatitis I presume, Dr Watson, with thirteen months of jaundice and no progressive histological changes in the liver

A man of sixty-four came in when he was jaundiced about two weeks, rather deeply jaundiced, with a four plus cephalin flocculation, increased thymol turbidity and a serum alkaline phosphatase of five Bodansky units We could not get a good history from him as he had been in a mental institution. With a profile such as he

presented, we thought he had hepatitis A liver biopsy showed the presence of cholangitis with some focal necrosis, not very much, and the pathologist thought that the changes were associated with an obstructive lesion He became much more deeply jaundiced in short order, the serum bilirubin rising to 51 mg. percent. He developed an hepatorenal syndrome with nitrogenous retention, oliguria, etc. Even though we now suspected extrahepatic involvement, the hepatitis element was so prominent we did not dare to interfere surgically. The predominant clinical picture was that of a severe hepatitis, which apparently was secondary to extrahepatic disease, namely, common duct stone, which was subsequently removed The first suspicion of extrahepatic disease was revealed by liver biopsy.

Liver biopsies are of value in following the course of liver disease. They are the best means of making sure that a hepatitis has completely disappeared and of following the development of chronic hepatitis or cirrhosis.

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A biopsy (Figure 11) was taken from a patient with sarcoidosis. He had scattered granulomata, which, though *not specific for* sarcoidosis, fit in well with the clinical picture A biopsy made ten months later showed fibrosis in addition to the sarcoidosis (Figure 12) Whether there is any relationship between the sarcoidosis and fibrosis, we are not sure Dr Gall believes that an early postnecrotic cirrhosis is present The relationship between granulomatous disease and cirrhosis has been hinted at in the literature, at least in the case of brucellosis(36, 37)

Liver biopsy is helpful in the evaluation of therapy in liver disease(38).

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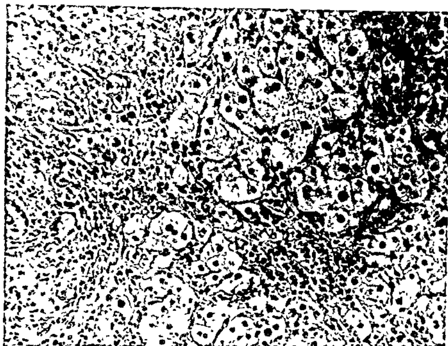


FIGURE 11 – A.I (C.E. 567)

Miliary Granuloma. Lobular architecture is distorted by irregular strands of fibrous tissue infiltrated by lymphocytes and plasma cells. At one edge, the margin of a tubercle containing two giant cells may be seen

shown in Figure 13. In studying the effect of therapy in fatty infiltration of the liver, it is of course necessary to have two biopsies, one as a control before starting specific therapy. You take a biopsy and put the patient on a hospital diet, and see what happens first on such a regime. Here the control biopsy showed the fatty infiltration to be the same. We gave her choline.

Watson. How long was the interval? How long was the control interval?

Schiff: About four weeks. We have seen fat disappear promptly on just the hospital diet without any choline, vitamin B supplements or other lipotropic agents.

Best: You mean without any added?

Schiff: I will stand corrected. We gave her four grams of choline a day, with a fairly well-balanced diet. We had her on that for six months. After six months the biopsy showed practically the same

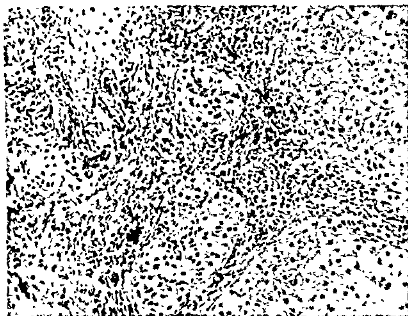


FIGURE 12 – A I (G E 698)

Same case as Figure 11, 10 months later. Fibrosis is prominent and there is now a well-defined micronodular structure Although inflammatory cells persist, tubercles are no longer evident in this specimen

amount of fatty infiltration We then raised the dose of choline to sixteen grams a day

Kinsell: Of what?

Schiff: Syrup of choline.

Gyorgy: Only about one-third?

Schiff We calculated the amount

Gyorgy One-third of that is choline.

Schiff: The preparation used was the syrup of choline dihydrogen citrate supplied by Flint Eaton & Co. of Decatur, Illinois.

Gyorgy: You gave 48 grams daily?

Schiff: We calculated that she was getting 16 grams a day. We had quite a time getting her to take it We biopsied her four months

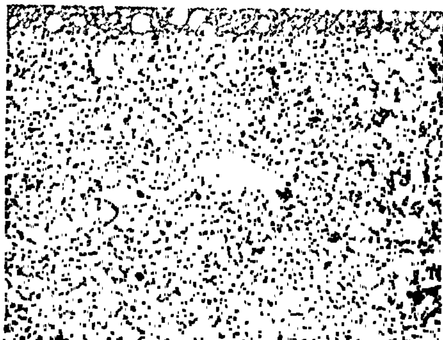


FIGURE 13 – J.M. (G.E. 138)

Severe Fatty Vacuolization. A severe grade of parenchymatous vacuolization with no evidence of liver cell necrosis.

later, and you see there appears to be a considerable decrease in the amount of fatty infiltration, at least we think so. (Figure 14).

We dropped the choline back to four grams a day, and four months later a liver biopsy revealed a marked increase in the degree of fatty vacuolization. I might say we have now observed this woman for over three years and she has not developed any histologic evidence of cirrhosis. Others have made similar observations(39).

Hartroft It appears to me that much of the fat in the section of liver illustrated is not *intra*cellular, but is actually *extra*cellular. Is not this lipid contained in pathological fatty cysts? In the photograph (Figure 13) is one such cyst with four nuclei in the wall which surrounds the contained fat. The appearance of this cyst closely resembles those we have found and reported in the livers of rats which develop experimental dietary cirrhosis due to a deficiency of dietary choline(40) Evidence based on experiments with rats with this type of fatty cirrhosis has suggested that the restoration of lipotropic factors to the diet fed the animals does not mobilize

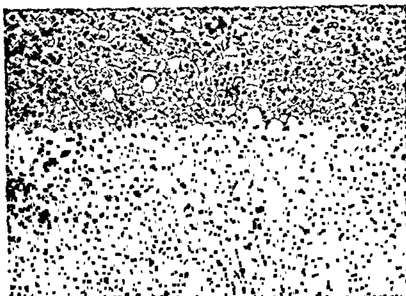


FIGURE 14—J.M. (C.E. 359)

Same patient (Figure 13), 10 months later. There is *a striking reduction in the amount of fatty vacuolization.* Liver cells show no evidence of distortion or disintegration.

*extra*cellular lipid in the fatty cysts nearly as rapidly as is the case when the hepatic fat is *intra*cellular.

Schiff: This woman has continued to show fatty vacuolization over three years.

Watson. She has remained obese?

Schiff: Yes. We tried to reduce her.

Watson. Cannot these areas be regarded simply as fat depots, just like fat in the abdominal wall or in the mesentery?

Best. I would not think so. They are pathological structures surely.

Hartroft: Lipid in pathological hepatic cysts of the type we have described is no longer inside liver cells. Perhaps cystic fat which is *extra*cellular is no longer directly subject to the metabolism of liver cells. Under these conditions, lipid in a fatty cyst in the liver may not be influenced by the lipotropic agents as rapidly as when the fat is *intra*cellular.

Neefe: Did she have any functional liver abnormalities?

Schiff: No. The profiles were repeatedly negative.

Hanger: I would like to know whether the liver became smaller when you gave those enormous doses of choline.

Schiff: She is a very obese woman. It is hard for me to answer that. It is a good question.

Hartroft: If one compares the lipid in a fatty liver cyst with that in adipose tissue, it would only be because the fat is not inside liver cells in either case. In neither instance does choline rapidly mobilize the lipid.

Schiff: We thought there was a little improvement in between We would not say she has gone on further.

Hartroft. Then choline has possibly arrested the progress?

Schiff: Yes.

Hanger: Are these not perhaps to be considered as foreign bodies and not as fat depots?

Hartroft: The fat will eventually leave the fatty cysts by unusual pathways of which we will speak later.

Schiff· Liver biopsy is also of value in the elucidation of the cause of enlargement of the liver.

Not infrequently we think a patient may have neoplasm of the liver and find he has cirrhosis. I am sure that this is everyone's experience.

A colored man of thirty-five complained of epigastric pain after meals for eight months. There was a history of tarry stools. An abdominal mass and an enlarged liver were present. The roentgenologist reported compression of the distal stomach and duodenum, probably by neoplasm. The liver profile was normal. We thought we were dealing with an intra-abdominal neoplasm with hepatic metastases.

The liver biopsy revealed the presence of tubercles. Because of the biopsy findings, tuberculosis was suspected. Laparotomy was performed, and the presence of abdominal tuberculosis was verified by lymph node biopsy There was no suspicion of granulomatous disease before the biopsy. I want to emphasize that Dr. Gall feels as others do, that these granulomas are not necessarily specific, and

that it may be impossible to differentiate the hepatic granulomas of sarcoidosis, syphillis, brucellosis, tuberculosis or of nonspecific granulomatous processes. If the clinician is made aware that a granulomatous disease is present, it is up to him to attempt to define its character.

Watson: In this connection the group at Leyden in Holland informed me that in doing liver biopsies in patients with advanced pulmonary tuberculosis, patients without any evidence of miliary tuberculosis in the ordinary sense, and they were surprised at the high number of positive biopsies for tuberculosis in the liver. Similar observations have been reported by Croxatto and Palermo(41).

Schiff: Was hepatic enlargement present?

Watson: I cannot give you the figures but I believe they were independent of any enlargement. They were doing transthoracic biopsies.

Knisely: One of the ideas on the initiation of tuberculosis developed by Preston Kyes, who died recently, a student of Paul Ehrlich, was as follows: One of the ways that tuberculosis gets in is by way of the digestive tract. The organism is carried to the liver The phagocytes digest the tubercle bacilli most of the time but not always Dr. Kyes demonstrated tubercle bacilli in liver phagocytes very early after inoculating the animals

Gyorgy· Was not L Calmette the first to claim that?

Knisely· I don't know.

Gyorgy It goes back fifty years.

Schiff Liver biopsy is also of value in the elucidation of hepatosplenopathies

We studied a young man of twenty, vaguely ill for eleven months, with twenty pounds weight loss He had been found to have an enlarged liver and spleen three months before The liver was down four finger-breadths There was a slight enlargement of the inguinal nodes and there were those who thought he might have Hodgkin's disease

In Figure 15, beautiful giant cells with Schaumann or pre-Schaumann bodies are shown This led to the clinical diagnosis of sarcoidosis, with which his course has been in keeping The value of liver biopsy in the diagnosis of sarcoidosis has been previously reported(42, 43).

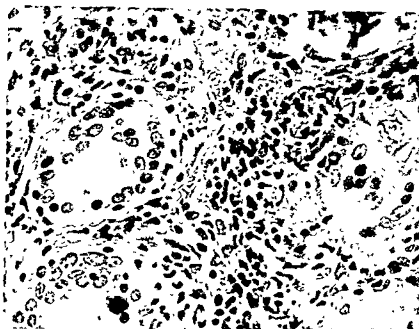


FIGURE 15—B W (C E 640)

Miliary Granuloma A high power view demonstrating a pleomorphic epithelioid exudate and several of the giant cells containing vacuoles and incl of nondescript character

A man of twenty-four was admitted with weakness, anore nausea, cramping abdominal pain, chills and fever of one mon duration. The liver was large, as was the spleen; a marked leukope existed Typhoid fever was suspected Liver biopsy revealed gra lomata with cells suggestive of the Dorothy Reed-Sternberg ty At the time of the biopsy, there was no peripheral lympadenopa About a month later, large inguinal nodes were discovered wh when biopsied, showed the typical changes of Hodgkin's disea

In a case of aleukemic lymphatic leukemia with enlargement the liver and spleen, liver biopsy showed leukemic cells in the tended sinusoids very beautifully and served to confirm the di nosis The bone marrow biopsy had not been satisfactory.

Liver biopsy is of value in the differentiation of extrahepatic a intrahepatic block in patients being considered for abdomi venous anastomotic operations.

A girl of 15 years had two recent hematemeses and a previo hematemesis at the age of eight She had a very large spleen. T

zinc sulfate turbidity was increased and she had a four plus cephalin flocculation reaction There was questionable bromsulfalein retention Intrahepatic block was suspected on the basis of the laboratory findings, but a liver biopsy was negative. She had an arteriovenous aneurysm between the splenic vein and splenic artery, and a splenectomy was performed

The liver biopsy is of value in the diagnosis of granulomatous disease

Giant cells were found scattered throughout the liver biopsy specimen of a patient with miliary tuberculosis who had no hepatic enlargement and who raised no sputum. In this case the liver biopsy furnished the first definitely confirmatory evidence of a tuberculous etiology.

Granulomatous lesions were present in the liver of a patient with a positive blood culture for Brucella suis Dr Spink and his associates(36) and Cazal(37) have well described these granulomata with their central zone of epitheloid cells and peripheral zone of lymphocytes.

A patient wit' [illegible]
How often this [illegible]
with secondary [illegible]
other granulomatous processes but, as frequently occurs in other granulomatous diseases in which the liver is involved, her liver was not enlarged and the liver profile was entirely normal. This emphasizes the importance of the biopsy in the detection of these lesions

In infectious mononucleosis, the biopsy may show the accumulation of the atypical lymphocytes in the sinusoids. Dr. Gall has seen this several times and believes that he can diagnose infectious mononucleosis from the liver biopsy

We have been surprised to find how often biopsy demonstrates the presence of neoplasm in the liver. We realize that as time goes on our experience may not be as striking as it has been up to now. For example, in 53 cases of proved neoplasm, which we have reported, liver biopsy demonstrated the presence of tumor in 41 of these(44) In five instances we had to go in a second time. In one instance we had to go in a third time So when we suspect neoplasm, particularly if the liver is nodular – in many of these instances the liver is not nodular – we repeat the biopsy. A negative biopsy,

course, does not exclude tumor. In one man with a huge nodular liver, biopsy done transthoracically was negative. We were nevertheless sure he had a neoplasm. Using the anterior approach we went in a second time right over a nodule. We took three specimens, and in only one small area in one of the three specimens was tumor tissue present! Dr. Stanley Dorst, our dean, amusingly remarked that it was like falling into a haystack and coming up without any hay! Incidentally, our batting average is just as high with the transthoracic approach as with the anterior approach. The liver is often much more extensively invaded by tumor than its gross external appearance might lead one to believe. Not infrequently, tumor nodules may be confined to the deeper substance of the liver, so that the liver surface may be smooth.

In Figure 16, the liver biopsy from a young person is presented This revealed a nonlipid histiocytosis.

I should stress the fact that needle biopsy of the liver may prove a very useful research tool. One can remove two or three pieces



FIGURE 16 – M P (G E 207)

Nonlipid Histiocytosis Embedded in a fibrous stroma are masses of closely packed mature histiocytes Nuclei are uniform and cytoplasm is free of inclusions

of liver tissue 2 or 3 cm. long and about 2 mm. wide. Various techniques may be applied to these depending on the investigator's interests.

Knisely: May I ask if it is possible to do some enzyme chemistry on some of these?

Schiff: Histochemical studies have been done We have been trying to do some micro-fat determinations Incidentally, we have not found the phosphatase stains of any value in the differential diagnosis of jaundice(45). We cannot tell obstructive jaundice from hepatitis by our phosphatase stains I think Sheila Sherlock and her associates(46) found more of a difference than we did

A short time ago I reported on a series of patients who had 574 biopsies(47). The clinical diagnosis was confirmed in 249, corrected in 98, and entirely unsuspected disease was revealed in 12. So in a little over 70 percent of our material, and naturally this will vary with the type of material used, the biopsy was a positive aid in diagnosis.

Biopsy was noncontributory to diagnosis in 92, misleading in 15 and the specimens were inadequate in 34, making a total of 141 or 28 2 percent of the 500 patients in whom it was of no aid in diagnosis Biopsy was most apt to be misleading in the presence of neoplasm by failing to reveal the tumor We have had two misses in postnecrotic cirrhosis. In nutritional or alcoholic cirrhosis, I don't think you will miss the lesion in a biopsy specimen We have not, as far as I know. In congestive failure, at least in cases of recent congestive failure in which the liver still appears to be enlarged, and in which there may be some abnormality in liver function tests, liver biopsy may prove normal I wrote Dr Bradley about this once We wonder if the blood is not squeezed out of the sinusoids at the time of biopsy and if the distention of the sinusoids as seen post-mortem may not be a post-mortem change.

Our overall percentage of failure to obtain an adequate biopsy specimen is now 4.8 percent One's ability to get adequate liver tissue increases with increasing experience. For example, our original percentage of failures was 40

In 46 patients who we thought had nutritional or postnecrotic cirrhosis, biopsy indicated that we were probably wrong in that 22 had fatty vacuolization, 11 had normal livers, 4 had hepatitis, 3 had neoplasm, 5 had biliary cirrhosis and one had obstructive jaundice

is present in the lymph The lymph from the intestine over a 24-hour period adds about twice the amount of esterified cholesterol as is contained in the total volume of circulating plasma. The normal intestinal mucosa and the normal liver each contains about 10 times the free cholesterol concentration of the plasma but only a little greater concentration of esterified cholesterol We do not know whether the liver regulates the esterifying function of the intestines or if the liver directly regulates the cholesterol composition of the blood.

Watson: When the cholesterol esters get down below 10 percent, it is fairly safe to say that there is a severe degree of liver insufficiency not incompatible with recovery but many patients die, especially when the value goes below 5 percent. That would imply that the gastrointestinal mucosa is also seriously injured, if your observations apply to human beings at all

Fremont-Smith Or the liver is destroying the cholesterol esters, is that possible?

Watson: That seems rather unlikely because we see the lowest values when the liver is diffusely necrotic

Madden· Are they receiving by way of the intestinal tract any cholesterol precursor material?

Fremont-Smith: They are not receiving much of anything At that time they are vomiting.

Watson What most of them are receiving is by vein.

Fremont-Smith: So the gastrointestinal tract would perhaps, not have a chance to manufacture it.

Popper. I would like to ask Dr. Watson a question We usually refer to the reduced percentage of cholesterol esters as an indication of severe liver cell damage. In obstructive jaundice one finds very commonly a low percentage with normal or even increased absolute values of cholesterol esters (in view of the markedly increased total cholesterol) even when only moderate degree of liver cell damage is present Should we pay more attention to the absolute value of cholesterol esters or to the percentage?

Watson. I think we ought to pay more attention to the absolute value If you take the average case of cancer of the pancreas, where there has been jaundice for any appreciable duration, the ester percentage is usually quite low It is not uncommon to find it between 20 and 30 percent The total cholesterol is as a rule ele-

In 16 patients who we thought clinically had metastatic neoplasm of the liver, 6 turned out on biopsy to have cirrhosis, 3 obstructive jaundice, etc.

In 11 patients who we thought were suffering from hepatitis, the biopsies showed cirrhosis in 5, obstructive jaundice in 3, etc.

In 9 patients thought to have obstructive jaundice, biopsy indicated hepatitis in 7, etc.

Looking at it in another way, in 112 patients proved by biopsy to have cirrhosis, the clinical diagnosis was incorrect in 20, or 18 percent, in 82 patients proved to have hepatitis by biopsy, 12, or about 14 percent, were thought to have obstructive jaundice.

We believe, therefore, that the liver biopsy possesses tremendous clinical value along the lines we have indicated.

Best. I have two questions. I must say I don't think any of us know the answers to them, and they are from the point of view of the experimentalist: Why are the cholesterol esters low in hepatitis?

Schiff· I would rather defer that to somebody else.

Best: There is nobody else to defer it to.

Fremont-Smith How do we know? Maybe there is.

Best: Has anybody any idea? Are they specifically low?

Watson· Isn't it related to the functional injury of the liver cell?

Best· I thought from Dr. Schiff's data that the esters were specifically low when the cholesterol was normal and the fat was normal

Watson. I assumed it was a matter of inability to esterify cholesterol.

Hanger. The esterase of serum is low, which probably parallels hepatic injury.

Bollman The explanation of the finding of low values for cholesterol esters is not simple. Dogs and rats fed free cholesterol have the major portion of the absorbed cholesterol esterified by the time it reaches the intestinal lymph. We therefore know that cholesterol may be esterified and passed on to the blood by the intestinal mucosa. Intestinal lymph from fasting animals or animals fed diets free of cholesterol contains free cholesterol in concentrations similar to that of the plasma but 30 to 50 percent more esterified cholesterol

is present in the lymph The lymph from the intestine over a 24-hour period adds about twice the amount of esterified cholesterol as is contained in the total volume of circulating plasma. The normal intestinal mucosa and the normal liver each contains about 10 times the free cholesterol concentration of the plasma but only a little greater concentration of esterified cholesterol. We do not know whether the liver regulates the esterifying function of the intestines or if the liver directly regulates the cholesterol composition of the blood.

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vated up to 250 or above 300, so one gets more information from the absolute values of esters than from the percentage. I don't know why that is. As a matter of fact, I have the impression, and I would like to hear more people comment, there is more lowering in patients with cancer causing obstructive jaundice than in cases of stone or benign obstruction.

Popper: I ask the question for the following reason: In early extrahepatic biliary obstruction the total cholesterol may rise to values above 300 or 350 mg. per 100 cc. At this time the ester percentage is normal; that means the esters rise also, for instance, to levels of 200 mg per 100 cc. or more. On this basis the ester percentage was originally considered an indication of hepatic damage. If, however, the obstruction persists for some time, the ester percentage starts going down very markedly. Shall we consider that a specific type of liver cell damage in these obstructive cases appears before the other hepatic tests reveal severe liver damage or does it mean that the esters stay constant but the free cholesterol rises due to biliary retention or increased formation? We are under the impression that with normal total cholesterol values severe liver cell damage is required to depress the ester percentage, whereas with high total cholesterol levels minor liver cell damage depresses the ester percentage

Hanger: The drop of cholesterol esters in long-standing obstruction is a very ominous sign and usually there is also a decrease of serum albumin The late stages of biliary cirrhosis are marked by similar changes Whenever there is an obstructive factor the total serum cholesterol is valueless unless an ester partition is done. The lower the ester percentage, the more definite the indication of hepatic damage. In our clinic, reliance is placed on absolute values. The idea of ratio or percentage of free to esterified cholesterol is really meaningless

Best: The other question I would raise is: What is the cause of hepatic coma?

Gyorgy. Good questions!

Schiff: I would like to defer on that one, too.

Best: I know nothing about it. Sometimes we know the causes of coma What do we know about hepatic coma?

Fremont-Smith Have there been any other instances of recovery from hepatic coma? Most have been in acute yellow atrophy, and

they have gone on to death Was there a recovery in the case which was reported this morning?

Schiff: Oh, yes.

Gyorgy: There are plenty of recoveries.

Schiff: We see that in cirrhotic patients.

Shorr. Isn't the speed rather amazing?

Watson. They recover after coma. That is not uncommon.

Fremont-Smith There are no residual cerebral aftereffects?

Hanger We had one cirrhotic patient with an Eck's fistula who tended to become comatose after the administration of ammonium salt or high protein diet. Is it not possible that increases in blood ammonium are depressing to the nervous system?

Fremont-Smith: Would that not provide an opportunity for experimental studies in animals? Has anybody seen hepatic coma in animals?

Watson You could produce it, of course, by severe damage

Gyorgy Even in so-called dietary necrosis.

Fremont-Smith It would be possible to do your kind of experiment, Dr Hanger, and get brain wave studies.

Hanger The patient cited was quite complicated Dr. Bollman has probably seen a thousand animals with that condition.

Bollman That is a little high You have to differentiate the two types of coma There would be an ahepatic coma such as the liverless animal will go into before it dies, and that differs quite markedly from the clinical hepatic coma The only other type of coma that I have ever seen in animals with rather damaged liver has been the toxic type which I cannot differentiate and say there is any specific liver factor in it at all Still that does not look like the clinical hepatic coma to me

Best I was telling Dr Ivy the other day, Dr Bollman, that Markowitz in extension of his work is now keeping his animals alive after complete ligation of the hepatic artery by giving streptomycin and penicillin In the work at the Mayo Clinic, all animals died if you did not give them antibiotics Dr Ivy said, "I tie the hepatic artery completely, and the animals don't die if I don't give them anything"

Bollman: They are both correct, strangely enough. This is an example of one of the points Dr. Fremont-Smith has often made that when two experiments or two experimenters fail to agree it is usually because the experimenters do not understand each other completely. Closer scrutiny of the experiments may bring out a difference which clarifies the situation and may prove a point of more value than those considered in the original experiments I am sure that Dr. Markowitz ligates several branches of the hepatic artery and I believe that Dr. Ivy ligated the main hepatic artery. If I am correct as to Dr. Ivy's experiments he has demonstrated that most dogs have enough collateral arterial circulation beyond his site of ligation of the hepatic artery. Dr. Markowitz's procedure acutely reduces the arterial flow to the liver and most dogs die about a day later because of the rapid development of anaerobic organisms in the liver If such animals receive penicillin the infection is prevented and after the first day or two the circulation of the liver has become adjusted so that the infection does not occur Dr Grindlay and I have injected radio-opaque material into the aorta of a number of dogs surviving (with penicillin) several days after ligation of the branches of the hepatic artery. In all these the surviving liver was found to have definite arterial blood supply and where portions of the liver were necrotic we were unable to demonstrate arterial circulation

Gyorgy. With penicillin?

Bollman· Penicillin will keep the animal alive but not the liver deprived of arterial circulation.

Best: That seems wrong to me If the liver fails, the animal dies

Bollman· It depends on the amount of surviving liver tissue and the extent of the areas of necrosis.

Best· Ivy's explanation was that he put his animals on a milk diet for two or three weeks, and the flora of the intestine changed, and even if the liver did become gangrenous the organisms in the liver did not cause the same change.

Bollman. That is rather surprising to me as I have thought that the Welch group of anaerobes which infect the liver were always present in the intestinal tract of dogs. If the infection is prevented for a day or so, most of the animals would survive unless extensive hepatic necrosis occurred In this field a large number of control animals operated in the same way by the same surgeon seems to be absolutely necessary

Best. We are getting off our subject perhaps.

Watson: There is one interesting observation recently that bears directly on the question of the genesis of the coma. Amatuzio and Nesbitt(48) at the Minneapolis Veterans Hospital, have been studying pyruvic acid in spinal fluid in patients with hepatic coma, and it is nearly always significantly increased I understand that normally there is no pyruvic acid demonstrable in the spinal fluid. In patients with hepatic coma there are quite frequently relatively large amounts, together with increased amounts of pyruvic acid in the blood.

Fremont-Smith Will that throw any light on the observation I made twice, many years ago, that the spinal fluid in severe hepatic injury may be yellow, may be tinted, and if it is tinted in these two cases the pigment disappears on exposure to light? Has anybody run into that or could it have anything to do with the pyruvic acid content?

Stetten It is hard to see how pyruvic acid per se could be a cause for coma

Watson I did not mean that They don't think it is the cause. It is simply an evidence of deranged metabolism in the brain

Neefe Also keto-glutaric, pyruvic and lactic acids may be elevated Dr Reinhold has been interested in this and, in the last two cases studied, marked elevations of all three acids were found. Thus there must be some intermediary metabolic defect

Knisely. May I ask Dr Bollman a question? In the animals which go into hepatic coma, are there known alterations in the blood chemistry caused by the absence of the liver, which absences cause the coma?

Bollman The answer is definitely no All of the substances we have investigated which are increased in the blood following complete removal of the liver are not toxic at these levels The deficiencies which follow removal of the liver also do not account for the failure of the animal

Gordon I would like to ask Dr Schiff a question that I think he can answer The question is relative to bile lakes Have you been able to check at autopsy just how widely spread throughout the organ or how close together these are? In other words, how likely are you to encounter them on a needle biopsy?

Schiff I am afraid I cannot answer that question, Dr. Gordon It is a very good question

Madden: May I ask Dr. Popper a question about his comments regarding the possible correlation of the stainable material in the cytoplasm, to the serum protein, or serum albumin levels? He seemed to find some basis for suggesting that a reduction in the amount of pyronine stainable material occurred concomitantly with the lowered serum albumin level, and I wondered how that might [illegible] labelled material in at least one case of cirrhosis.

Popper: I want to stress again that the relation between protein synthesis and cytoplasmic pentose nucleic acid is not proven, however, the circumstantial evidence for this relation is constantly increasing. If we find a low serum albumin and the hepatic cells depleted of pentose nucleic acid, we still have no right to assume that this indicates reduced protein formation by the liver. There is not only a turnover of serum protein but also of pentose nucleic acid involved. The histologic findings are in keeping with both possibilities: reduced protein formation as well as rapid turnover. As mentioned before, in cirrhosis large nucleoli and a small rim of basophilia around the nucleus can be seen in a cell the cytoplasm of which is otherwise depleted of basophilic bodies. This could be interpreted as evidence for an accelerated attempt at new formation of pentose nucleic acid, possibly related to the markedly increased protein formation, or as a depletion of pentose nucleic acid and depressed protein formation. As in any histochemical study, we always see only one stage and not the course of events In this connection I would like to point to an example which shows the reverse relationship, Rich and Berthrong(49) have found in certain conditions, such as infections which damage the liver cells, large clumps of basophilic material of pentose nucleic acid nature. One could entertain the hypothesis that this indicates a blocked utilization of the pentose nucleic acid. If this assumption is right, one should get definite changes in the liver cells after plasmaphoresis From what I have heard, the cells are depleted of basophilia after plasmaphoresis I assume that the nucleoli are large. We would like to investigate this interesting point because we may have here morphologic evidence for increased protein formation with rapid turnover of pentose nucleic acid I say this again with all the caution which histochemistry requires

Neefe. I would like to raise one or two points regarding biopsies In the past year or so we have had occasion to do biopsies on

asymptomatic young men whom we happened to study as part of a survey, the objective of which was to determine whether residual liver damage occurred frequently following acute hepatitis. These persons came in at our request and not because they were ill Thus, they were presumably healthy young people. In some, suggestive evidence of mild hepatic disturbance usually minimal elevation of bromsulphalein or perhaps an increase in urine urobilinogen, or occasionally a positive flocculation reaction has been found Liver biopsies have been obtained from a few persons of this type and certain findings, the significance of which I certainly do not understand, have been noted Three observations in particular have interested me. One is so-called brown pigmentation I wonder if anyone here has been impressed with the occurrence of this pigment It apparently is not iron positive and is not bilirubin. Some of these sections show quite a bit of this fine brownish colored pigmentation in the cells In several biopsies from the above cases, an excessive amount of fat in the Kupffer cells has been reported to me by Dr. William Ehrich The Kupffer cells were distended with fat. Another finding has been the presence of considerable iron positive pigment in the cells. This presumably represents hemosiderosis. These findings then have been noted in biopsies from persons who are asymptomatic. Maybe I should not say that Are they physiologic or are they abnormal? Has anyone information concerning the significance of these observations? I should mention though that all of these people have had some mild abnormality in one or another of the hepatic tests. Otherwise we would not have done the biopsy, because we feel we have to have some excuse for having done it in the event of a complication

Popper: I believe the appearance of pigment in livers of healthy young people has some significance Lipofuchsin pigment has been demonstrated by Lucké(50) after complete recovery from viral hepatitis I do not think you will find it in specimens taken at random I am more doubtful about the significance of fat in Kupffer cells The normal Kupffer cells contain fat, as shown for instance by Levine(51), and this fat content varies markedly, so I would not attach too much significance to it Iron pigment in small amounts is not infrequently seen in the liver I would like to add, however, one series of observations We occasionally see in biopsy specimens of persons in whom we would not suspect significant functional alterations in the liver, changes such as inflammatory cells in the portal triads of small focal necroses in the parenchyma We have been concerned about the diagnostic significance of these changes

and whether or not their presence can be related to the presenting disease or condition. To answer this question, we studied autopsy specimens from 93 healthy young soldiers who died instantaneously, mostly in airplane crashes, (their *Dissé spaces* were not visible) and compared them with 219 cases of persons who died also rather rapidly, but still after a considerable agonal period. Especially in *the first group we can be sure that they were clinically* well and did not have even a respiratory disease or else they would not have gone up on a mission. In 37 7 percent of the first and 32 4 percent of the second group occasional small focal necroses with segmented leucocytes were noted in the parenchyma A slight increase in portal cellularity was seen in 24 7 percent of the first and 27.4 percent of the second group. Considerable infiltration, sometimes even including segmented leucocytes in one or another portal space was noted in 38.6 percent of the first and 44 8 percent of the second group. Fat infiltration was observed in about 2 percent of each group. I do not know what these changes mean, but they surely do *not imply that the person is sick* Whether these changes mean a present or a preceding anicteric hepatitis is surely conjectural I think one of the great pitfalls in the study of liver *biopsy is that a perfectly normal person may have changes in the* liver, the significance of which we do not know.

Fremont-Smith We have the same thing in the kidney. All kidneys, normal ones of adults, have dying and dead glomeruli. A certain amount of dead and dying glomeruli is normal for a normal kidney. It shows that the use of the word "normal" is confusing and we find we have to say normal *with respect to* something. It seems to me that you have made a very good point that *there is a certain amount of pathology*, which if looked at comparing one cell to another is pathological, but in toto may be normal with respect to what we can call normal That probably holds for every *organ.*

Popper: If such changes occur in livers of perfectly normal people what right do we have to consider them as morphologic evidence of a protracted hepatitis or even as caused by hepatitis at *all*, if we should find them in persons who happen to have had clinical hepatitis some time previously?

Schiff. We have seen some degree of periportal infiltration in individuals who are in all other respects normal, and it has raised *the question, the point,* [illegible] opper made: In cases that have had a previous hepatitis, [illegible] idual p [illegible] l hepatitis have

any significance at all? We have seen it a number of times. We did not think there was any indication of any disease

Hanger: Have any of you used glycogen stains in your studies? I have noted that glycogen is not demonstrable in certain parts of injured hepatic cells For example, the bile stained areas often contain no glycogen It raises the possibility that some of the disturbances of the diseased liver cell are focal, in which case the whole cell may not be affected, but a segment of it is enzymatically deranged

Popper: We often performed glycogen stains or better said, the periodic acidfuchsin sulphurous acid technique of McManus(52), which in the liver cell stains almost only glycogen. It has not assisted us very much However, one point you just made I would like to support In viral hepatitis, intracellular foci are noted which are free of basophilia or glycogen. If they grow to involve the entire cell they become a Councilman body. Benda, Gerlach, Rissel, and Thaler(53) have considered them precursors of Councilman bodies and connected them tentatively with the presence of the virus.

Neefe. I wanted to raise the question again with respect to the periportal infiltrations A number of pathologists have told me that in routine post-mortem material in persons who are supposedly free of liver disease, on all clinical grounds at least, that such infiltrations are fairly frequent However, it has also been said that if you take biopsies at operation, a biopsy secured at the beginning of the operation is quite different than if it is taken at the time, as most surgeons do, just before they are ready to close the abdomen It is said that such periportal infiltrations will appear even in such short a time as during the period of an operative procedure It does make one wonder whether some of those things might not develop in the same way during the agonal state as they do during an operative procedure, and whether or not we can actually transfer that experience to biopsy material secured during life I think perhaps one of our greatest needs is a larger series of normal biopsies, a thing that is very difficult to arrange

Popper We also have found in surgical liver biopsy specimens a high incidence of focal necroses and cellular infiltration

Best Unless there is an urgent question, I think we should go on

Knisely It is the same question about the rates of fixation of cells if you are going to look at glycogen That is said most humbly and in friendship.

Best: We all realize this, Dr. Knisely.

Knisely: I am a little defensive about this as I lived in a comparative world. The fixation of glycogen is not too easy. Absolute alcohol is one of the fixatives used for glycogen. This substance diffuses into cells. It moves the glycogen in the cell. We have had histology class-room teaching sections fixed by freezing, and some fixed in fluid fixatives. The fluid fixatives almost uniformly pushes glycogen to one side of the cell (see Maximow and Bloom(54), Figures 10 and 11).

Hartroft: When sections of frozen-dried material are compared with sections of similar tissue fixed with alcohol, is there any evidence that the glycogen in the latter instance has moved not only from its original position within the cell to some other region of the same cell, but also from one cell to another?

Knisely: Within one cell in my experience.

Popper: Glycogen moves during alcohol fixation into the tissue spaces and even into the vessels, as shown by Meixner(55).

Knisely: That is easily believable.

Hartroft: Is it any more justifiable to draw conclusions as to the distribution of glycogen within the liver lobule than it is to do so in regard to the distribution within specific areas of a single cell, on the basis of the usual alcohol-fixed preparations? Should one employ only tissue prepared by freezing and drying to avoid fallacious results?

Knisely: It not only moves, but it becomes unstainable.

Hartroft: You are referring to the results obtained with the freezing and drying method?

Knisely: That method holds it homogeneously. Then by looking at the amount of redness, you have to try to say how many milligrams of glycogen are present!

Davies: May I raise one point here about the cellular infiltrates in the portal triads. This is often written off as an age-change but seeing, as I have done, a great number of liver specimens from small children I have repeatedly noticed that mononuclear infiltration usually goes with an episode of fatty infiltration. When you get infections on top of the fatty infiltration then you get infiltrates of polymorphonuclears plus the mononuclears; in patients with ancylostomiasis or other parasitic disease you see eosinophilic infiltration, and these cellular infiltrates persist for a long time, perhaps

throughout the life of the African. I never see a liver without considerable amounts of these cellular infiltrates far in excess of what one sees in Europeans. I think that there is some significance in this. I am not going to say that it is pathological, but I don't think it ought to be written off as an age-change or one of those things seen too frequently to bother about. It may be an indication of a past episode of damage to the liver

REFERENCES

1 POPPER, H Significance of agonal changes in human liver *Arch Path. 46, 132 (1948)*

2 ELIAS, H A re-examination of the structure of the mammalian liver. I Parenchymal architecture *Am J Anat* 84, *311 (1949).*

3. RÖSSLE, R.: Entzuendungen der Leber, in Henke F. and Lubarsch, O., *Handbuch der speziellen pathologischen Anatomie und Histologie* Berlin J. Springer, 1930, (vol 5 part 1)

4 EPPINGER, H, KAUNITZ, H, and POPPER, H · *Die seröse Entzuendung* Vienna J. Springer, 1935

5 POPPER, H Über seröse Hepatitis *Wien klin Wchnschr. 49, 207 (1936)*

6 WALDSTEIN, S S, and SZANTO, P B Accuracy of sampling by needle biopsy in diffuse liver disease *Arch Path* 50, *326 (1950)*

7. FRANKLIN, M, POPPER, H, STEIGMANN, F, and KOZOLL, D D. Relation between structural and functional alterations of liver. *J. Lab. & Clin Med* 33, *435 (1948)*

8 POPPER, H, STEIGMANN, F, MEYER, K.A, KOZOLL, D D, and FRANKLIN, M Correlation of liver function and liver structure. Clinical applications. *Am J Med. 6, 278 (1949)*

9 POPPER, H, STEIGMANN, F, and SZANTO, P. B.· Quantitative correlation of morphologic liver changes and clinical tests. *Am. J Clin. Path* 19, *710 (1949).*

10 GILLMAN, J and GILLMAN, T · Anoxia and liver with special reference to shock and chronic malnutrition *South African J M Sc.* 13, *11 (1948)*

11 POPPER, H, WALDSTEIN, S S, and SZANTO, P B Correlation of clinical features of cirrhosis of liver with findings on biopsy *Am J Clin Path* 20, *724 (1950)*

12 WALDSTEIN, S S, POPPER, H, SZANTO, P B, and STEIGMANN, F Liver cirrhosis Relation between function and structure *(in preparation)*

13 MOSCHCOWITZ, E · Laennec cirrhosis; its histogenesis, with special reference to role of angiogenesis *Arch. Path. 45, 187 (1948)*

CLINICAL ASPECTS OF HEPATIC VASCULAR PHYSIOLOGY

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The hepatic circulation is large and vital to the body economy. Approximately one-quarter of the cardiac output passes through the liver each minute, permitting it to operate importantly in systematic hemodynamics Both arterial and venous blood perfuse the liver under high and low pressures respectively, necessitating special vascular arrangements that are as yet imperfectly understood. The hypotensive portal venous system is particularly susceptible to disturbance by disease. Interference with hepatic blood flow often leads to ascites, splenomegaly, and the development of collateral venous channels apparently as the result of portal-hypertension. Though such derangements of hepatic vascular physiology are readily apparent to the clinician, normal functional changes and minor disturbances are not easily detected or evaluated. This is attributable in the main to the inaccessibility of the liver and the

the Evans Memorial Hospital in Boston, has already been described in detail to this group(2). This paper will be devoted to an examination of the validity of this method and to consideration of data which have emerged from its use in certain clinical studies.

Validity of the Method: Estimated hepatic blood flow (EHBF) is calculated on the basis of a value for hepatic removal of bromsulfalein (BSP) and a measurement of hepatic BSP uptake as follows: EHBF = R/A-V where R is BSP removal in mg. per min and A-V is the difference between BSP concentrations in peripheral arterial or venous blood and in hepatic venous blood in mg per ml. The value of hepatic BSP removal is taken as equal to the rate of BSP infusion where the peripheral blood concentration of BSP remains unchanged Corrections for changing levels may be made on the assumption that BSP, being protein-bound, is contained

46. Sherlock, S, and Walshe, V.: Hepatic alkaline phosphatase; histological and microchemical studies on liver tissue in normal subjects and in liver and in bone disease. *J. Path. Bact* 59, 615 (1947).

47. Schiff, L : The clinical value of needle biopsy of the liver. *Ann. Int. Med.* (*in press*).

48. Amatuzio, D. S, and Nesbitt, S : A study of pyruvic acid in the blood and spinal fluid of patients with liver disease with and without hepatic coma. *J. Clin. Investigation* 29, 1486 (1950).

49. Rich, A. R., and Berthrong, M.: Evidence for the presence of ribonucleic acid in the cytoplasmic bodies that appear in the hepatic and adrenal epithelial cells of man in acute infections. *Bull. Johns Hopkins Hosp.* 85, 327 (1949).

50 Lucké, B · Structure of liver after recovery from epidemic hepatitis. *Am. J. Path.* 20, 595 (1944).

51. Levine, V.: Normal fat content of the Kupffer cells. *Arch. Path* 14, 345 (1932).

52. McManus, J. F. A : Histological and histochemical uses of periodic acid stain. *Technol.* 23, 99 (1948).

53 Benda, L., Gerlach, F., Rissel, E, and Thaler, H : Über Untersuchungen zur Frage der Virusatiologie der Hepatitis epidemica. *Arch f. Virusforsch.* 4, 89 (1949).

54. Maximow, A. A, and Bloom, W.: *A Textbook of Histology* Philadelphia and London: W. B. Saunders Co, 1948 (5th ed), Figures 10 and 11 (p 13).

55 Meixner, K.: Das Glykogen das Leber bei Verschiedenen Todesarten. *Beitr. gerichtl. Med.* 1, 222 (1911).

SECTION II

CLINICAL ASPECTS OF HEPATIC VASCULAR PHYSIOLOGY

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during the time of study solely within the vascular system (or the blood volume). Hepatic venous blood is obtained by venous catheterization. The concentration of BSP is kept on a relatively constant value below 2 mg. percent by constant intravenous infusion *using a Bowman pump**

Obviously a method of this kind entails a number of assumptions that require careful validation and that must approximate the truth. In early discussions(1) we were most concerned with the fact that the hepatic venous sample is removed from a vein draining only a portion of the liver since the various hepatic veins empty separately into the inferior vena cava and mixed hepatic venous blood is unobtainable We have placed the catheter in different parts of the liver and different hepatic veins in the same individual on several occasions in order to determine whether BSP concentra*tion varies greatly from place to place when the peripheral con*centration is maintained at a constant level. A difference of as much as 20 percent in extractions (BSP arterio-venous concentration difference divided by arterial or peripheral venous concentration) was observed on two occasions, in the remainder the values were in much better agreement (Table I). Samples obtained close to the mouth of the vein appeared to be most divergent, and it seemed likely that regurgitation of blood from the inferior vena cava might be responsible For this reason a practice has been made of taking the hepatic venous blood from a position deep in the *right lobe of the liver and assuming it to be representative of mixed* venous blood. This difficulty must introduce a large error and may account in part for the range of values observed in any series. Moreover, movements of the catheter during respiration may introduce variation in any given individual and must be prevented if possible

TABLE I
BROMSULFALEIN EXTRACTION AT DIFFERENT SITES IN THE LIVER*

Subject and Diagnosis	Sex	Age	S A M²	PBSP mgm. per 100 ml	EBSP percent	RBSP mgm per min	EHBF ml. per min
J.M Syphilis	M	25	2 00	1.20	86 2	6 2	1,090
				1 03	74 6	6 0	1,420
				0 91	66 2	6 2	1,860
N.O'N Syphilis	F	35	1.60	1 07	47 4	4 8	1,590
				1 24	49 4	4 6	1,290
				1 23	39 4	4 8	1,700
				1 08	49 1	4 8	1,530
V B Syphilis	F	19	1.53	1 37	69 1	4 9	940
				1 48	46 0	4 9	1,315
				1 50	43 0	4 9	1,370
				1 41	55 6	4 9	1,140
A B Syphilis	F	23	1 61	0 78	77.5	5 0	1,440
				0 62	83 9	5 0	1,660
				0 65	91.5	4 7	1,370
R S Syphilis	F	20	1.58	1.48	64 1	6 0	1,210
				1.26	58 5	4 8	1,200
				1 30	59 0	4 6	1,150
				1 31	62 9	5 0	1,180
				1 22	71 2	4 9	1,080
J S Arthralgia	M	51	1 78	1 53	59 0	5 1	1,090
				1 50	50 3	5 0	1,280
E McK Cirrhosis	F	67	1 80	2 15	15 8	1 9	980
				2 21	13 1	1 9	1,150
O H Cirrhosis	M	38	1 65	1 09	22 9	2.5	1,995
				1 13	24 0	2 4	1,760

* Each value is based upon a blood sample obtained at differ... and left hepatic veins PBSP is the ... removal ... blood flow

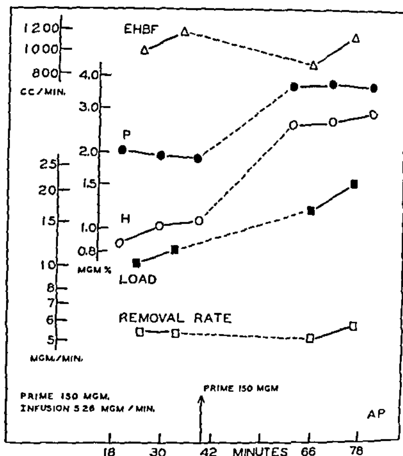


FIGURE 1

... estimated
hep...
ven...
by...
... n peripheral and
... tic concentration
... hed Despite the
... for EHBF The
... P and EHBF,
... BSP.

by return of dye at the higher blood level through collateral channels of any size would result in a further underestimation of the removal rate and a fall in EHBF. Under these circumstances enterohepatic circulation of dye would not affect estimation of EHBF unless hepatic venous concentrations exceeded peripheral

values, this has never been observed under the established conditions. However, Lorber and Shay(3) have found demonstrable blood levels of BSP after duodenal instillation of 2 to 5 mg. BSP per Kg., suggesting the poss [illegible]
tion of dye from the bile i [illegible]
intravenous administration. [illegible]
or insignificant when BSP is given in doses of 2 mg. or less per Kg., which is higher than the usual total dose in EHBF measurements.

We have failed to find significant concentration of BSP in the blood of six patients with cirrhosis and successful portocaval anastomosis 30 to 60 minutes following administration of 450 to 500 mg. of BSP by mouth. It is possible that the discrepancy is attributable to destruction of BSP in the stomach. Finally, determination of BSP in portal* and peripheral arterial blood obtained simultaneously is five subjects revealed equal concentrations. Consequently, enterohepatic circulation of BSP seems an unlikely cause for error in the method.

The use of the BSP infusion rate at the concentration equilibrium as a measure of hepatic removal is predicated on the belief that extrahepatic removal is insignificant when the blood level is kept below 2 mg percent. Since extrahepatic removal appears to be a function of concentration, whereas hepatic removal appears to be limited and independent of concentration above 2 to 4 mg. percent (see Figure 1) it follows that the extrahepatic escape of dye from the circulation at high concentrations may be far in excess of the hepatic removal rate. Thus, Cohn and his co-workers(4) found that less than 0 34 mg of BSP was removed from the blood each minute in the eviscerated dog at levels of 1.75 to 2.65 mg. percent, whereas more than 2 4 mg. was removed per minute in the intact animal At higher levels BSP removal in the eviscerated animal exceeded hepatic removal in intact animals(5) These findings stress the need for working at lower concentrations of BSP. Dr Archibald Macpherson and I have carried out studies of BSP removal in five eviscerated dogs, attempting to maintain the plasma levels constant at low levels by reducing the infusion rate just prior to hepatectomy. Despite all precautions the plasma level always mounted sharply, owing to the reduction in BSP removal Two of these studies are presented graphically in Figures

* Portal blood was obtained through the courtesy of Drs Blakemore and Fitzpatrick either at operation or several days postoperatively through a small plastic catheter placed in the portal vein carried out through the incision, and left in this position for several days for infusion of heparin

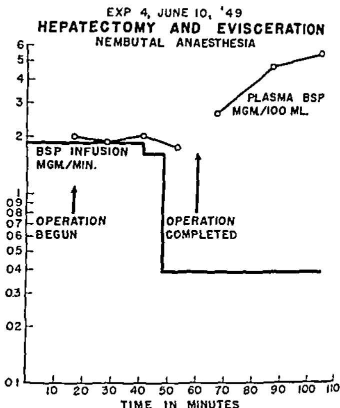


FIGURE 2

Effect of hepatectomy and evisceration on BSP removal in the dog An infusi

increased sharply, because removal of BSP almost ceased with hepatectomy.

2 and 3 It can be seen that total BSP removal decreased marked following hepatectomy – to less than 5 percent of the contro and higher plasma levels were necessary to achieve these value These results have encouraged us to use the method even in th presence of liver damage where hepatic removal is reduced sinc the error thus introduced appears to be smaller than that resultin from sampling of blood from a single hepatic vein.

Hepatic Vascular Reactivity in Normal Man: Studies of th behavior of the splanchnic circulation in normal subjects followin administration of epinephrine, histamine, and glucose, assumptio of the upright position, and application of an abdominal binde

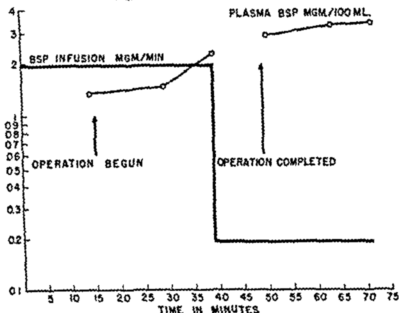


FIGURE 3

Effect of hepatectomy and evisceration on BSP removal in the dog The infusion of BSP was reduced to a much slower rate (0 2 mg per minute) than in the experiment shown in Figure 2 and the BSP concentration tended to stabilize after an initial elevation Removal here also has virtually ceased

have shown a wide range of vascular reactivity. Some of these results have been reported to you in previous conferences(2, 6). Evidence of vasoconstrictor (during orthostasis) and vasodilator (following epinephrine) responses have been elicited. In addition, we have recently obtained evidence of augmented splanchnic flow during the pyrogenic reaction(7) and of diminished flow during the action of various anesthetic agents(8).

In association with Dr Neal J. Conan, determinations of EHBF were made in twelve normal human subjects before and after intravenous administration of typhoid vaccine In all, the BSP plasma level rose sharply during the chill and fever phase in association with diminished hepatic BSP extraction, indicating a marked reduction in hepatic removal This phenomenon occurred

even when fever was prevented by premedication with antipyretics The elevation in BSP concentration was so rapid and the extraction so reduced in four individuals that satisfactory determination of EHBF was impossible In the remaining eight individuals EHBF increased though it did not always remain high, apparently because arterial pressure tended to fall. An alarming shock-like state supervened in several instances.

The data obtained in a study of subject A. DeJ. are presented in Figure 4. This 27-year-old white male was given one gram of antipyrine in divided doses during the 24 hours prior to study. Typhoid vaccine (0.1 ml.) was administered intravenously 46 minutes after 150 mg. of bromsulfalein had been given as a priming dose, and bromsulfalein infusion started at a rate of 5.62 mg. per minute. Approximately 35 minutes after administration of the pyrogen, the infusion rate was slowed to 3.75 mg. per minute in

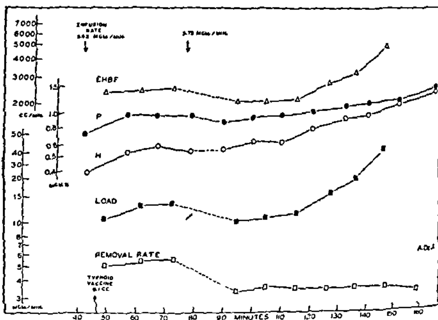


FIGURE 4

Effect of the pyrogenic reaction on EHBF Symbols as in Figure 1. Following

cased loading as a result of augmented
The body temperature did not change
throughout this study owing to preliminary medication with antipyrine.

order to avoid the usual elevation in BSP concentration. It can be seen that the concentration rose slightly during the reaction (which was afebrile) despite the reduction in infusion rate. Bromsulfalein removal decreased despite increased loading (calculated from blood flow and peripheral plasma BSP concentration). The EHBF increased sharply as the BSP arteriovenous difference and extraction percentage fell. In the final period BSP extraction was less than 10 percent, and EHBF could not be determined The removal rate remained constant throughout, following the initial fall, from which it may be inferred that extraction decreased as a result of both diminished transfer of BSP and augmented blood flow This constancy of removal despite a steadily increasing load suggests that a transfer limit had been reached. Mason and his associates(9) have found much higher values for dogs than those observed (about 5 mg per minute, normally) in the course of these studies on man. Since they have made their measurements at very high plasma levels of BSP it is possible that a relatively large extrahepatic BSP removal vitiates their measurements. The fact that maximal hepatic BSP removal decreased during the pyrogenic reaction does not imply a reduction in functioning liver mass, but indicates rather a specific interference with hepatocellular BSP pickup perhaps independently of other activities. This inference finds support in the fact that hepatic galactose removal increased in the one subject, hepatic oxygen uptake rose in the three, and glucose output increased in the two subjects in which these measurements were made

In contrast to the changes observed during the pyrogenic reaction, thiopental and cyclopropane has been found to cause a fall in EHBF in five of six subjects. Bromsulfalein removal and extrac-tion varied during anesthesia, changing very little in three, and falling off sharply in the others Hence, we are unprepared to say definitely whether hepatocellular dysfunction develops in patients receiving these agents Certainly the change in EHBF occurs independently of change in arterial pressure and it may be inferred that there is vasoconstriction in the splanchnic vascular bed.

An attempt has been made to determine whether this response can be blocked by high spinal anesthesia (to D2 or D3). Since the blood pressure tends to fall under these circumstances the results are somewhat difficult to interpret. However, in one patient in whom blood pressure remained unchanged, EHBF decreased after thiopental In two others pretreated with ephedrine (20 mg. intra-

venously and 30 mg. subcutaneously) to sustain the arterial pressure, EHBF fell following induction of anesthesia with cyclopropane. Thus, it may be concluded tentatively that high spinal anesthesia appears to have no effect upon EHBF independently of a fall in blood pressure, and does not seem to block the action of anesthetic agents. These studies are still in the preliminary stage, and further investigation may change our present opinion.

In general, the behavior pattern of the hepatic vasculature resembles that of the peripheral circulatory system. During the pyrogenic reaction the cardiac output is increased and blood flow through the kidneys and skin greatly augmented(10). During anesthesia or anoxia, on the other hand, there is renal vasoconstriction(8) and cardiac output may fall if anesthesia is sufficiently profound(11). Thus, the hepatic circulation appears to operate in conformity with the requirements of the systemic circuit under conditions that elicit widespread vascular readjustments.

Hepatic Vascular Reactivity in Cirrhosis: Measurements of EHBF have been made in 33 patients with cirrhosis due to various causes in association with Dr. F. Ingelfinger at the Evans Memorial Hospital, Boston, and Dr. A. Groff, Dr. G. Bradley and many others, at Presbyterian Hospital, New York. These values are plotted against figures for the hepatic extraction of BSP in Figure 5 All determinations of EHBF made in subjects with a BSP extraction of less than 10 percent have been excluded from this figure Measurements were made in most instances when the BSP concentration was changing no more than 0 005 mg percent per minute. It can be seen that EHBF fell below the normal range in the majority of instances Extraction, likewise, was usually impaired. There was no obvious difference between individuals with alcoholic, postnecrotic, syphilitic or parasitic cirrhosis(12) and it may be concluded that the hepatic blood flow is usually reduced in cirrhotic disease It is difficult to assess these figures in relative terms since a good measure of functioning liver tissue is wanting. However, measurements of hepatic oxygen uptake (Figure 6) have revealed reduced hepatic venous oxygen concentrations and augmented hepatic arteriovenous oxygen differences in the majority of patients in whom they were measured It seems reasonable to assume that the difference between oxygen concentrations in the hepatic vein and hepatic artery (based on measurements of brachial or femoral arterial oxygen content) is dependent in the main upon hepatocellular activity, since studies made by Myers(13) on

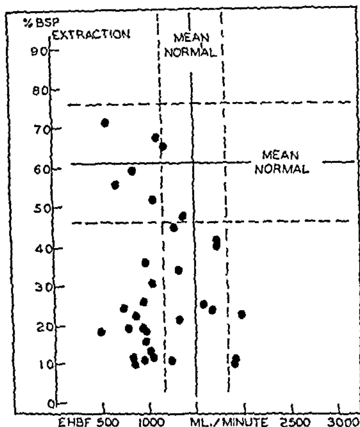


FIGURE 5

Estimated hepatic blood flow and bromsulfalein extraction in cirrhosis of the liver

umbilical vein blood and in our laboratory on blood obtained directly from the portal vein indicate low oxygen removal by the gastrointestinal tract. The reduction in oxygen A-V difference therefore implies slower perfusion of hepatic tissue or hepatic ischemia.

Studies of EHBF and hepatic oxygen extraction before and after portocaval anastomosis have been made in collaboration with Drs A. H. Blakemore, A. Macpherson, and A. Gammeltoft. Satisfactory data were obtained in seven individuals and are presented

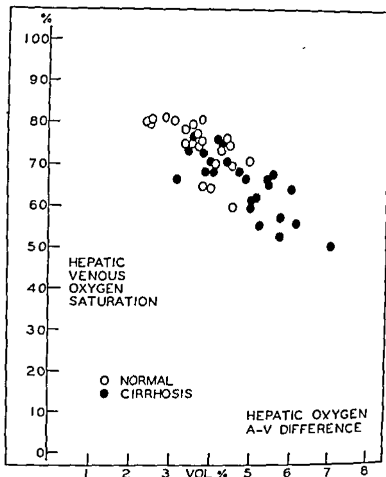


FIGURE 6

Hepatic oxygen arteriovenous difference and hepatic venous oxygen saturation in cirrhosis of the liver.

in Table II. In every instance, EHBF fell postoperatively in association with an increment in oxygen A-V difference. This response may be interpreted as evidence for a reduction in hepatic blood flow as a result of the fall in portal venous pressure after successful anastomosis The diminished flow of blood accounts for the fall in hepatic venous oxygen content provided hepatic oxygen consumption remains unchanged and gastrointestinal oxygen uptake is not great. There is no reason to expect a change in hepatic oxygen consumption, and reference has already been made to the evidence for a high portal venous oxygen content. The changes

observed appear to indicate that the liver receives a large proportion of its oxygen supply by way of the portal vein. It is possible, of course, that vasoconstriction develops in the hepatic circuit following the shunting procedure, but it seems unlikely.

We are now engaged in a long-term study of the vascular reactivity in the cirrhotic liver. Dr. J. D. Myers(14) has found

TABLE II

EFFECT OF PORTOCAVAL ANASTOMOSIS ON EHBF AND HEPATIC OXYGEN UPTAKE IN CIRRHOSIS*

Subjects	PRESHUNT			POSTSHUNT		
	EBSP	EHBF	Oxygen A-V Diff	EBSP	EHBF	Oxygen A-V diff
	percent	ml. per min	ml. per 100 ml.	percent	ml per min.	ml per 100 ml.
K.N.	12	1,025	3 5	38	940	4 7
P.N	18	1,885	3 6	46	880	6 8
P.P.	15	1,080	6 1	38	710	8.4
T.K.	42	1,725	3 7	36	1,490	4 9
P.McC.	32	1,030	4 6	41	615	8.4
N D	34	1,315	4 5	37	1,025	5 2
A R	26	940	4 3	43	535	6.3

* Abbreviations as in Table I, Oxygen A-V difference is the difference between oxygen concentrations in hepatic venous and peripheral arterial blood

that EHBF increases somewhat following intravenous administration of salt-poor concentrated human serum albumin This seemed to be a safe tool to use in assessing the capacity of the hepatic vasculature to undergo vasodilation Dr Gammeltoft and I have given 50 grams of albumin intravenously within a 20-minute period to seven patients with cirrhosis The data in Table III show that EHBF usually rose. Indeed the increment in this value was much greater than that observed by Myers(14) in normal subjects It seems unlikely that this is an artefact produced by hemodilution since it occurred very rapidly at least 30 minutes before any change in hematocrit occurred Moreover, BSP extraction decreased and BSP concentration rose One would expect that an organic disorder characterized by interference with blood flow and oxygen supply would evoke a maximal compensatory vasodilation, but these results suggest that vasoconstriction, in addition to structural interference with flow, may play a role in producing hepatic ischemia in

decreased output with decrease in rate of flow. That agrees precisely with some basic physiology which has largely been lost from the American literature Back about 1912, Professor August Krogh and Professor Lindhard measured their own personal cardiac outputs When these athletically untrained professors were at rest, their cardiac output was about 3 5 to 4 liters of blood per minute(15, 16, 17). As these professors went into severe muscular exercise their cardiac output went up to about 21 liters per minute or a little more. Since then, Dr. Hovy Christansen has found the cardiac output to go up to as high as 35 liters per minute in untrained muscular athletes The next sharply significant finding was that the sudden increase in cardiac output which occurred as these men went from complete rest to severe muscular exercise began before there was time for blood to come from the exercising muscles of the back legs to the heart. From this, Professor Krogh deduced that there must be a large store house for blood in the body capable of emptying blood into the heart quickly From his current knowledge of anatomy, he said that the store house could only be one with a large capacity and connected in shunt with the rest of the circulatory system He deduced that this could only be the liver and branches of the portal vein bed, and of course, we today would add the spleen

It is known that in frogs, mice, guinea pigs, rabbits and rhesus monkeys there is a sphincter-like outlet valve at the point where each hepatic sinusoid joins the central vein of the lobule(18) It is known that in dogs, the hepatic veins are powerfully contractile(19) Both types of outlet valve or flow control mechanisms may be present in all vertebrate species as far as we can now know.

The control of the outflow of blood from the liver into the inferior vena cava is without doubt one of the major determinants of cardiac output The rate of outflow from the liver into the vena cava determines circulating blood volume, venous pressure, and cardiac output The heart cannot pump any blood it does not get, and it pumps very nearly all the blood it does get as fast as that blood is put into it. (For a detailed account of this, see reference 18) All of the data you have presented agrees with the above concepts precisely and like the work of Krogh and Lindhard, the experiments were done on people, which makes it wonderful

You presented one more thing which fascinated me utterly. One of the problems that we have in medicine is how fast can the body

kill bacteria. Drinker and Shaw(20) showed that the liver is the main organ for phagocytizing material from the blood stream. The histologists sometimes say that the spleen is the main organ, but that is attempting to judge in terms of phagocytes per microscope field. The work of Drinker and Shaw was done by actual chemical determination of the total amount removed in each of several organs, and the liver does most of it.

There is an old problem in the medical literature about how does fever help the patient. You have shown that there is a sharp increase in the rate of flow of blood through the liver with the rise in temperature and increase in cardiac output, which must mean that there is more blood per minute or per hour going through the liver. There is a young physician at Duke University by the name of Samuel Martin, who is measuring the number of bacteria or the percentage of drop in count of bacteria between the blood entering the liver and leaving it. I think he is using rabbits mostly, but that may be wrong. This statement is without his permission. He finds that sometimes as much as 80 percent of the entering bacteria are removed. So with the passage of time one would get 80 percent of 80 percent, like the mathematics of paying interest out of a fixed fund, resulting in a rapid decrease in the numbers of circulating organisms.

The percentage varies depending upon the kind of bacteria, the kinds of surfaces they have and some other factors. But the total numbers removed per hour must depend upon the total number of phagocytes which are present in the liver, the bacteria present [illegible] biological factors, the percentage of [illegible] through the liver. [illegible] key facts between life and death. In raising [illegible] the increase in rate of flow through the liver it would seem, other things being equal, must increase the numbers of bacteria removed per hour or per day. This becomes a very precious point You may feel a little defensive about your method, but the method is certainly overwhelmingly valid for showing that.

It seems to me that [illegible] eat deal o[illegible] must begin to move from asking [illegible] can the[illegible]s ingest bacteria" to asking "How fa[illegible] phagocy[illegible] the bacteria?" In all the reading I h[illegible] have [illegible] of anyone who showed how [illegible] a [illegible] would

lead directly to a more rapid rate of death of pathologic organisms. This seems to me to be a key point in medicine.

Bradley: I think there is one point that might be stressed, and that is the fact that this occurs without a change in body temperature

Knisely: If you raise the patient's temperature without putting in particular vaccines, what happens?

Bradley: As far as we can tell by disappearance of bromsulfalein the same thing apparently does happen.

Knisely: If you put them in a hot box?

Bradley: In the hypertherm the bromsulfalein removal from the blood is decreased(21). Measurements of EHBF under these circumstances are impossible at present.

Knisely: *May I add one more thing: If you have striated muscles* at rest, and the human patient consists of 20 to 40 percent striated muscle by weight according to Vierordt's tables, as far as we know there would be very little blood flow through the muscles(22). If the muscles are at rest, and there is high cardiac output, there must be a fast flow through the liver. These would become the basic conditions for the maximum possible rates of carrying particles to the hepatic phagocytes.

Best: Are there any other questions?

Handler. Did you have any change in the plasma protein concentration after you gave albumin?

Bradley: We have a measurement of that. There is a definite but slight increase in plasma protein concentration

Watson With only 50 grams in the cirrhotic who has a lower serum albumin do you not observe a transitory rise?

Bradley Yes, a small transitory elevation

Knisely The question of changes in arterial and portal flow was raised earlier It may be well for the record to question the validity of old figures by Burton Opitz that a third or a quarter of the flow is arterial and the rest venous Burton Opitz measured the rate of blood flow through the hepatic artery of one dog, with a Ludwig's two-chambered stromuhr, then measured the rate of flow through the portal vein *of another dog* He added the two and calculated

kill bacteria. Drinker and Shaw(20) showed that the liver is the main organ for phagocytizing material from the blood stream. The histologists sometimes say that the spleen is the main organ, but that is attempting to judge in terms of phagocytes per microscope field. The work of Drinker and Shaw was done by actual chemical determination of the total amount removed in each of several organs, and the liver does most of it.

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The percentage varies depending upon the kind of bacteria, the kinds of surfaces they have and some other factors. But the total numbers removed per hour must depend upon the total number of phagocytes which are present in the liver, the bacteria present per cc. of blood and two physiological factors, the percentage of bacteria removed on one passage, and the rate of blood flow through the liver. This brings us precisely to the point which is one of the key facts between life and death. In raising the body's temperature the increase in rate of flow through the liver it would seem, other things being equal, must increase the numbers of bacteria removed per hour or per day. This becomes a very precious point. You may feel a little defensive about your method, but the method is certainly overwhelmingly valid for showing that.

It seems to me that a great deal of immunology must begin to move from asking "How fast can the phagocytes ingest bacteria" to asking "How fast can the phagocytes digest the bacteria?" In all the reading I have done, I have never before read of anyone who showed how the increase in a temperature of the body would

lead directly to a more rapid rate of death of pathologic organisms. This seems to me to be a key point in medicine.

Bradley: I think there is one point that might be stressed, and that is the fact that this occurs without a change in body temperature.

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the proportion. That is the source of the current concepts that from one-fourth to one-third of the flow through the liver is arterial, the rest portal. He never did both measurements on one dog The figures of Soskin, Essex, Herrick and Mann(23) go as high as 90 percent for either vessel.

Bollman: The total blood flow to the liver varies with the cardiac output, but the proportions of portal and arterial blood do not remain constant. In anesthetized dogs the hepatic artery carried from 11 to 83 percent of the blood to the liver(24) Simultaneous observations of inflow and outflow indicate that the liver alternately stores and discharges part of its blood. The blood flow to the liver and from the liver is subject to rhythmic variations. The average blood flow is only an average, and we know that the blood flow through most of the sinusoids is usually intermittent.

Gordon: I have a couple of points I would like to bring up, Dr. Bradley. The first is for my own information. I don't quite understand why it is necessary to assume from the data that there is dilation of the vessels in the liver. It seems to me that all of the findings and the data could be explained simply on the basis of a more rapid blood flow.

The second point relates to what happens to the blood flow in normal animals that have a portocaval shunt.

Bradley Increased blood flow through any circuit implies either diminished resistance to flow or increased perfusing pressure Since we have no evidence of change in the character of the blood or in blood pressure it follows of necessity that blood flow increased in these studies as a result of diminished resistance or vasodilatation We have no data on the behavior of blood flow in animals with a portocaval anastomosis.

Schiff: Dr Bradley, is there any difference in the estimated hepatic venous blood flow in patients with cirrhosis with ascites or without ascites?

Bradley We have not been able to discern any difference, but we do not have sufficient data to make a definitive statement.

REFERENCES

1. BRADLEY, S E, INGELFINGER, F J, BRADLEY, G P, and CURRY, J J: The estimation of hepatic blood flow in man. *J Clin Investigation* 24, 890 (1945).
2. BRADLEY, S E Liver function as studied by hepatic vein catheterization. *Livery Injury* Hoffbauer, F W, Editor Trans Fifth Conf. New York Josiah Macy, Jr Foundation, 1946 (p 38).
3. LORBER, S H, and SHAY, H.. Entero-hepatic circulation of bromsulphalein *J Clin Investigation* 29, 831 (1950)
4. COHN, C, LEVINE, R, and STREICHER, D The rate of removal of intravenously injected bromsulphalein by the liver and extra hepatic tissues of the dog *Am J Physiol* 150, 299 (1947)
5. COHN, C, LEVINE, R, and KOLINSKY, M Hepatic and peripheral removal rates, in the dog, for intravenously injected bromsulphalein *Am J Physiol* 155, 286 (1948)
6. BRADLEY, S Effect of posture and exercise upon blood flow through the liver *Liver Injury* Hoffbauer, F W, Editor Trans Seventh Conf. New York Josiah Macy, Jr, Foundation, 1948 (p 53)
7. BRADLEY, S E, and CONAN, N J Estimated hepatic blood flow and bromsulphalein extraction in normal man during the pyrogenic reaction *J Clin Investigation* 26, 1175 (1947)
8. PAPPER, E M, HABIF, D V, and BRADLEY, S E Studies of renal and hepatic function in normal man during thiopental, cyclopropane and high spinal anesthesia *J Clin Investigation* 29, 838 (1950)
9. MASON, M F, HAWLEY, G, and SMITH, A Application of the saturation principle to the estimation of functional hepatic mass in normal dogs *Am J Physiol* 152, 42 (1948)
10. BRADLEY, S E, CHASIS, H, GOLDRING, W, and SMITH, H W Hemodynamic alterations in normotensive and hypertensive subjects during pyrogenic reaction *J Clin Investigation* 24, 749 (1945)
11. GOODMAN, L, and GILMAN, A *The Pharmacological Basis of Therapeutics* New York The Macmillan Company, 1941
12. BRADLEY, S E, INGELFINGER F J, GROFF, A E, and BRADLEY, G P Estimated hepatic blood flow and hepatic venous oxygen content in cirrhosis of the liver *Proc Soc Exper Biol & Med* 67, 206 (1948)
13. MYERS J D The hepatic blood flow in Laennec's cirrhosis, with an estimate of the relative contributions from portal vein and hepatic artery *J Clin Investigation* 29, 836 (1950)
14. MYERS J D Observations on the excretion of bromsulphalein. *J Clin Investigation* 28, [illegible] (1949)

15. KROGH, A, and LINDHARD, J.: Measurements of the blood flow through the lungs of man. *Skand. Arch. f. Physiol.* 27, 100 *(1912)*.
16. KROGH, A.: On the influence of the venous supply upon the output of the heart. *Skand. Arch. f. Physiol.* 27, 126 *(1912)*.
17. KROGH, A.: The regulation of the supply of blood to the right heart, with a description of a new circulation model. *Skand Arch. f. Physiol.* 27, 227 *(1912)*.
18. KNISELY, M. H, BLOCH, E. H., and WARNER, L.: Selective phagocytosis, I. Microscopic observations concerning the regulation of the blood flow through the liver and other organs and the mechanism and rate of phagocytic removal of particles from the blood. *Det Kongelige Danske Videnskabernes Selskab,* Biologiske Skrifter, Bind IV, Nr. 7, København *(1948)*.
19. THOMAS, W. D, and ESSEX, H. E.: Observations on the hepatic venous circulation with special reference to the sphincteric mechanism. *Am. J. Physiol.* 158, *303 (1949)*.
20 DRINKER, C. K., and SHAW, L. A.: Quantitative distribution of particulate material (manganese dioxide) administered intravenously to the cat. *J. Exper. Med.* 33, *77 (1921)*.
21. CONAN, N S.: Personal communication.
22. KROGH, A.: *The Anatomy and Physiology of Capillaries.* New Haven' Yale University Press, 1929.
23 SOSKIN, S, ESSEX, H. E., HERRICK, J. F., and MANN, F. C.: Mechanism of regulation of blood sugar by liver. *Am. J. Physiol* 124, *558 (1938)*.
24 GRINDLAY, J. H, HERRICK, J F., and MANN, F. C: Measurement of blood flow of liver *Am. J. Physiol* 132, *489 (1941)*.

SECTION III

STUDIES OF HEPATIC LYMPHATICS

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I THOUGHT perhaps a discussion of this subject would fit in with the discussion of circulation to the liver. The work that I want to show was done by Dr. L. E. Johnson who has been working mainly with Dr. F. C. Mann and Dr. J. H. Grindlay in the study of the lymphatics of the liver. As perhaps I have mentioned before, one of the reasons that we are interested in the lymphatics of the liver was the observation that the flow of lymph from the liver is tremendously increased in the presence of experimentally produced cirrhosis, and also tremendously increased if we produce hepatovenous congestion by partial occlusion of the vena cava above the liver, in which case ascites develops. With this ascites there is tremendous increase in flow of lymph from the liver; the increased flow of liver lymph develops before ascites is present In the dog the liver lymph normally would be somewhere around 200 to 300 cc. of lymph a day, which amount carries with it a large amount of protein, about equivalent to between one-half and one-third of the total circulating plasma protein. In cases of cirrhosis when this lymph flow is greatly increased, the lymph then drained from the liver contains anywhere from 3 to 4, up as high as 10 times, as much as the total circulating protein So it becomes startling We got interested in lymphatics, and there were several questions that we wanted to know about lymph drainage of the liver. One of the first thing was: How intimately associated were the blood supply, the bile ducts, and the lymphatics, and could we demonstrate actual lymphatic drainage of the sinusoids of the liver? I think I can demonstrate most of our findings by means of lantern slides.*

The pig's liver has each lobule neatly delineated with a little connective tissue so that most lobules show a section as being hexagonal. The portal branches surround the lobule and the central vein shows in the center of the lobule. After retrograde injection

* Unfortunately, illustrations from these colored slides are not available for inclusion in the Transactions. – Editor.

of India ink into the liver lymphatic, sections of the liver show the ink very near the portal branches surrounding the lobule and in the longer lymphatics accompanying the blood vessels. No ink is found within the lobule. If there are lymphatic spaces between the sinusoids they are not injected by this technique.

Best: Where do you pick up the lymph channel to inject?

Bollman: The liver is retracted upward and the major liver lymphatic can usually be seen in the mesentery just below the liver. After considerable experience one can readily find the lymphatics from the liver. I have been surprised at how blind one can be We spent a long time looking for liver lymphatics in animals and were unable to see them. We then remembered McMaster's work delineating lymphatics with dyes. A small amount of Evans blue injected into the substance of the liver appears in the lymphatics within a few seconds and the blue lymphatics are easily seen. After seeing the blue lymphatics a few times the uninjected colorless lymphatics which had been previously invisible are readily apparent and it is difficult to understand why we did not see them before For injection the lymphatic is followed back as near as possible to the liver and a small plastic tube inserted and tied into the lymphatic. The retrograde injection is made slowly at very low pressures because the lymphatics rupture easily Dr Johnson injected several hundred rats and had only a few good preparations.

The blood vessels and the lymphatics may be injected with neoprene or vinyl acetate and casts of the vessels obtained when the tissue is removed by corrosion. When this is done the lymphatics are seen to follow the portal vein and we are impressed with the extensive lymphatic system.

Hartroft. Did you employ a special photographic technique? The effect is remarkable for it is almost stereoscopic.

Bollman: The photograph was taken through the dissecting microscope With greater magnification one can see how intimately the lymphatics follow the portal vein. Fine branches may be seen which accompany the portal vein probably to each liver lobule. It is obvious that not all the lymphatics are injected in any of these preparations and many more are present than can actually be seen. I want you to remember that the portal vein was directly injected but that the lymphatics were injected retrograde. The

flow of lymph is away from the liver opposite to that of the portal vein.

Some lymphatics also follow the hepatic veins By injecting lymph stained with Sudan IV retrograde under low pressure the pink stained lymphatics may be seen following and surrounding the hepatic veins Some of the lymphatics are seen in the walls of the hepatic veins. In some places it might appear that the lymphatics drained into the veins, but I am sure that this is not the case. In every instance where the lymphatic injection material did appear in the vein a definite rupture of the lymphatic could be demonstrated In sections of liver, after the lymphatics have been injected with India ink, many of the smaller blood vessels may be seen entirely surrounded by India ink contained in the lymphatics All of the ink is definitely enclosed in lymphatic vessels and many such areas are seen around the small blood vessels Within the liver lobule no ink is seen in the area from the portal capillaries to the central vein If there is a lymph channel between the sinusoids of the liver, it is not open sufficiently to be injected by this method If, as with the sinusoids, some are open intermittently, then some of the open lymphatic channels should be injected, but they are not. If the lymphatics penetrate the lobule there must be some sort of a valvular mechanism which prevents retrograde injection beyond the periphery of the lobule Only in rare sections was there any suggestion of the injected material appearing to be in a lymph space between the sinusoids and in each of them there was either an obvious artefact or serious doubt that the injected material was not actually in another plane and was actually in one of the larger lymph vessels. In each of Dr Johnson's lymphatic injections there were thousands of sinusoids which were not adjacent to any of the injected material so that the finding of the injected material in a single space was carefully investigated and usually was definitely found to be spurious

The lymphatics are also intimately associated with the bile ducts and the numerous channels almost completely surround the finer branches of each duct India ink injected retrograde into the lymphatics may be clearly seen in sections of the liver. In the small biliary ducts, the ink is seen just below the epithelium of the duct so that any diffusion from the duct would of necessity be almost completely surrounded by small lymphatic channels.

Best Does that suggest the lymph picks up something from the bile ducts?

Bollman: It does show that the biliary ducts have intimate and adequate lymph drainage. Any substance which would diffuse out of the bile duct would be surrounded by lymph capillaries which could easily pick up the extra biliary material. This intimate relationship between tissue spaces and the various thin walled vessels certainly provides a mechanism for maintaining equilibrium conditions in the liver. Materials could easily be transferred into the pool of tissue fluid from the arterioles, portal vein, bile capillary, hepatic vein or lymphatic, and from the hepatic cells. The nature and the amount of materials diffusing away from the tissue spaces into each of the above structures would follow the equilibrium relations between the tissue fluid and each of the intimately associated structures. You are all familiar with the fact that bile pigment appears in the hepatic lymph within a minute or two after obstruction of the biliary duct. Bile pigment also appears in the liver lymph during our procedure for cannulating the liver lymphatics of rats or dogs under ether anesthesia. In this procedure the surgical manipulation of the liver is not very great but bile pigment may continue to appear in the lymph for an hour or two while the animal is recovering from the effects of the operation.

Watson: The photographs are quite convincing with respect to regurgitation jaundice and permeability of the intrahepatic biliary tract due to injury. For instance, some bile could easily get across to the lymphatics and then into the blood. It is a beautiful demonstration of that relationship to me.

Bollman: When the lymphatics of the liver are injected retrograde with blue latex and the portal vein injected with white latex, the extensive and intimate association of the lymphatics with the portal vein are clearly demonstrated after corrosion of the tissue so that only the injected vessels remain in the specimen We are impressed with the volume of the lymphatics and wonder why so little lymph flows out of this large system of vessels. Again, it appears that the lymphatics are the drains from a mechanism for fluid and solute exchange

Davies: You have not demonstrated any finer channel than that we have seen around in the portal triad?

Bollman: That is right.

Davies: No connection at all with any part of the lobule itself?

Bollman: Only on rare occasions have we seen sections which

would suggest a connection within the lobule and in each of these we either proved it to be an artefact or strongly suspected that it was an artefact.

Best: Does it not seem rather surprising? I always thought of the capillaries being the part where the lymph channels would really start. You seem to get these lymph vessels around the larger vessels

Bollman: Yes.

Hanger: How does protein get in? Where does it come from?

Bollman: I still feel that the lymph does drain from the sinusoids and the lymph does contain almost as much protein and similar protein as does the plasma. This indicates to me that the sinusoids are quite permeable to protein, but that sufficient membranes are present to prevent the passage of cellular elements of the blood.

Hanger: Is there no evidence of valves in the lymphatic system?

Bollman: Very definite valves are present in the extrahepatic lymphatics

Best: You mean you overcome them when you do your retrograde injection?

Bollman: The injections must be made close to the liver and sometimes a valve must be pierced

Tarver: Why don't you attribute the results to failure of the retrograde injection method?

Bollman: I would certainly like to see a method for the direct injection rather than the retrograde method What we have been saying is that the retrograde injection only demonstrates the lymphatics back to the hepatic lobule and the only question is why the injection does not proceed further Retrograde injections of the hepatic vein extend for the most part into the sinusoids and we postulate a valvular or sphincteric mechanism of the sinusoids to account for those not injected Either the spaces within the lobule have too small an exit to permit inflow of the injection mass or a valvular mechanism is present Either choice brings you back only to the fact that the spaces are not injected.

Best I think Dr Knisely should make some comment.

Knisely: This is an old anatomical problem, and the pictures you present are in strict agreement with a paper by Lee(1) and the problem has been stated exactly. The question is whether the

Hartroft: They drain to the upper group of abdominal lymphatic nodes?

Bollman The liver lymphatics enter lymph nodes in the gastrohepatic omentum and this enters branches of the lymphatics from the upper portion of the intestine.

Hartroft. Do these lymphatics associated with the centrolobular veins not follow the hepatic vein, then?

Bollman: Some do, but I believe that only a small amount of lymph passes from the liver in this way.

Watson: In respect to Dr Popper's question about the dog, it might be pertinent to introduce a little historical note Some of you are perhaps acquainted with the book by Saunders on the liver written in 1809 It is really remarkable from the experimental standpoint. Saunders first points out that von Haller in about 1760 showed that if there were a very subtle increase in pressure on the common bile duct one could quickly detect bile pigment in the serum of hepatic vein blood On the basis of that, von Haller was the first to promulgate the idea of regurgitation jaundice. Then Saunders mentions that he repeated these experiments in dogs, by simply increasing the pressure in the common duct very slightly, and quickly observed that the lymphatics, or as he called them in those days, the absorbents, the absorbents draining the liver became yellow He was able to trace this regurgitation of bile from the liver via the absorbents very quickly Then he repeated von Haller's experiments and found that he could also demonstrate a return of bile into the blood of the hepatic vein, so his conclusion was that when there is increased pressure in the common bile duct there is a regurgitation of bile both by way of blood and by way of the lymph, but that the regurgitation of bile by way of the lymph occurs earlier and to a more marked extent than it does by way of the blood. I think those were really remarkable experimental studies that have largely been lost sight of. More recently the observations of others(5, 6, 7) indicate in general an earlier regurgitation via the lymphatics.

Best: If this early regurgitation is by way of the lymph, and if the hepatic vein lymphatics drain into the abdominal lymph ducts, I don't see how the appearance of bile pigments in the hepatic vein can be explained on the basis of these lymph channels.

Watson: That, I think, was assumed to be a direct regurgitation into the blood.

Best: You said that lymph was first?

Watson: That was first, and the sequence has been studied with thoracic duct fistulae more extensively. More recently, in the studies of Gonzales-Oddone(7) it was quite clear that the bile appeared first in the lymph of the thoracic duct before any increase in the blood could be demonstrated. However, that statement is not based on direct examination of the hepatic vein blood, but rather on blood out in the periphery, and the dilution factor has to be taken into account

Hanger: In some cases of extrahepatic obstructive jaundice the portal triads appear edematous. Don't you think that extra hepatic pressure on lymphatic channels emerging from the liver may cause "elephantiasis," so to speak, of the liver? It is due to this perhaps, as you pointed out, Dr. Schiff, that it is sometimes difficult to differentiate long-standing obstructive jaundice from cholangitic infiltration.

Hartroft: Have you any preparation, Dr Bollman, which shows the injection of lymphatics around the central veins? What is the pathway by which lymph from vessels near the central veins reaches the upper group of abdominal lymph nodes, which, you stated earlier, occurs?

Bollman: The injection of the lymphatics around the central vein shows plainly Unfortunately I did not bring a good section to illustrate this All of these preparations have been made by retrograde injection of the lymphatics draining caudal from the liver and the fact that the lymphatics following the hepatic vein are injected, indicates the free anastomosis between the lymphatics of the liver This is one of the reasons I believe that almost all of the lymph from the liver drains downward

Hartroft In sections, liver parenchyma is nearly always found separating portal vessels from central ones One wonders just where lymphatics would cross from one region to the other to reach the upper abdominal lymph nodes.

Popper. Is there not a large lymphatic network in Glisson's capsule?

Bollman The capsule is quite rich in lymphatics which follow the blood vessels from the capsule. The capsular lymphatics are enlarged and more prominent in cirrhotic livers

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Hartroft: They drain to the upper group of abdominal lymph

Bollman: The liver lymphatics enter lymph nodes in the gast hepatic omentum and this enters branches of the lymphatics fr the upper portion of the intestine.

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Knisely: In so far as I know the history, I think that Dale and his associates pointed out the dilatation of the veins in the intestines in dogs following histamine, and said that the great splanchnic dilatation, increasing the splanchnic bed, was one of the main causes of cardiac output failure in histamine-shocked dogs. That work was done long before there was the current degree of understanding of the presence of hepatic outlet constrictor mechanisms and of their response to histamine. The first detected response to histamine was dilatation of the splanchnic vessels. Later it was found that the valves down stream could close and back up pressure in them.

We are now faced with the problem whether the branches of the portal vein dilate in response to histamine directly touching them, or whether the sphincters shut off down stream from them and thus cause dilatation I am inclined now to believe the latter.

Bradley: When does engorgement take place?

Shorr: Only during the evolution of the hyporeactive or irreversible phase of shock. In the initial phase of shock, which is characterized by compensatory peripheral vascular adjustments through blood loss, the liver is firm and ischemic and scarcely bleeds when incised

Bradley. The portal radicals are not engorged.

Shorr. Not during the initial stage. Indeed, in the unanesthetized animal, exposed to graded hemorrhage, the compensatory vascular adjustments occur throughout the syndrome even with the most profound degrees of hypotension after bleeding The hemorrhage may be so great that the animal will die of ischemia, yet the liver will be firm and ischemic In the unanesthetized animal, therefore, the hyporeactive phase does not occur after graded hemorrhage and the liver does not get engorged.

Knisely: All our experience would agree.

Bradley Does engorgement occur just prior to death?

Shorr. No Engorgement begins with the development of the hyporeactive phase as indicated by the depression of mesenteric capillary reactivity and increases progressively After the extreme hypotension which is required to produce irreversibility by graded hemorrhage in the anesthetized animal has lasted from 90 to 120 minutes, profound engorgement has occurred This engorgement is

Knisely: I think valve mechanisms are almost certainly present in all vertebrate animals. Personally, I don't believe the circulatory system can function without hepatic outlet valves of some sort. Hydraulically, it would seem impossible for the system to work without them.

Then there is a third type of shock which we have initiated in dogs without hemorrhage. It may be worth while to put the method in here. Two dogs are used Let us call one a test dog and the other a tissue donor. Anesthetize both. Do a laparotomy down the mid-line of the linea alba in the test dog Then take some striated muscle from the tissue donor and this can be done very quickly, so as to minimize the possibility of the formation multiplying toxic substances from bacteria. Make a fine pulp of the striated muscle and put piles of it beside the test dog Then lift the intestines and mesenteries of the test dog out over the pulped striated muscle. The test dog can be opened with very little blood loss, and with no more stimulus to the nerves than there is in any such laparotomy. Thus, this experiment starts out with minimal hemorrhage and with minimal stimulus to nerves. Then, with a stereoscopic Leitz binocular microscope focused on the small arteries, capillaries and venules of the intestine and mesentery one sees that all of the blood coming down the arteries is fluid and all of the blood going back in the veins is precipitated, agglutinated. With the passage of time the whole circulating blood changes to thick sludge, and the blood then goes much too slowly. The small veins then begin to get anoxic (stagnant anoxia) and begin to leak, and finally plug. As they begin to leak, fluid is lost from the small leaking vessel from the circulatory system, and the masses that plug are lost from the circulation, and each mass that plugs a small vein blocks one path from the arterial system to the venous system Only after enormous numbers of small vessels are so plugged is there a fall in venous return and a fall of arterial pressure.

I don't know for sure now, but I would guess that that set of mechanisms would leave the liver sinusoids stuffed with masses of agglutinated red cells The fact that they were agglutinated would not show up in ordinary sections.

There are at least three separate mechanisms, which can cause shock, and that is what we are working with these days.

Shorr. Would it be necessary to postulate a stricture if you had a dilator mechanism, with an increasing intrahepatic pressure?

Knisely. In so far as I know the history, I think that Dale and his associates pointed out the dilatation of the veins in the intestines in dogs following histamine, and said that the great splanchnic dilatation, increasing the splanchnic bed, was one of the main causes of cardiac output failure in histamine-shocked dogs. That work was done long before there was the current degree of understanding of the presence of hepatic outlet constrictor mechanisms and of their response to histamine. The first detected response to histamine was dilatation of the splanchnic vessels. Later it was found that the valves down stream could close and back up pressure in them.

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further increased when such animals are transfused. The transfused blood apparently is pooled in large measure in the liver and increases the engorgement.

Knisely: Dr. Fine and his group at Beth Israel Hospital in Boston told me, and I am not sure whether it has been published, that when they took blood from a dog and reinjected it into the same dog, that blood did not go back in the circulation. The reservoir outlet control mechanisms had gone into spasm

They had evidence that the contractile mechanism was spasm of the hepatic veins. When they had a cannula in the hepatic vein, and when the vein went into spasm, it would hold the cannula so tightly that it could hardly be pulled out. That is direct mechanical evidence. I would think there are two significant points to this: One, that the hepatic veins did remain in spasm; further, in an animal with low blood volume retransfused with its own blood, and retransfused carefully, the outlet mechanism did not open as *teleologically* it should have The mechanisms of the animal did not go back into physiological operation.

Shorr: You say that blood did not find its way into the liver?

Knisely I cannot say whether it did not enter the liver. Perhaps it was in the portal vein bed and spleen alone, perhaps in the substance of the liver, too I strongly suspect that the liver filled up, but the hepatic outlet mechanisms stayed shut That is suspicion, not knowledge

Shorr: This is in the animal that would be considered irreversible?

Knisely: That would be the danger of it.

Best. A little adrenalin would open them up.

Knisely The problem is how to get the adrenalin into the hepatic veins when they are shut tightly If the adrenalin has to touch the walls of the vessels or their linings, it would be hard to get the adrenalin to these places to cause the vessels to dilate. If that could be done, it should work well

Shorr. I should imagine that you might be able to get adrenalin into the liver by perfusing it directly into the portal vein or hepatic artery. However, I should like to recall the experiments of Fine on the direct perfusion of the portal vein in dogs which have been made irreversible to transfusion When the transfusion via the portal vein is initiated fully after the development of irreversibility,

these animals still prove unresponsive to transfusions. In the light of our experiments, such animals would be liberating VDM (ferritin) even though oxidative conditions were restored.

Watson: What is the evidence of hepatic vein valves in the human being?

Knisely: Three papers by Krogh, in 1912,(11, 12, 13).

Watson: You are talking about sinusoidal sphincters in man to explain Krogh's finding?

Knisely: If present, yes From Krogh's work we know there is something in man which can contract. But our anatomical knowledge is incomplete for man. We know that the hepatic veins contract in dogs(10) and we know that in a series of species (frogs, mice, guinea pigs and rhesus monkeys) sinusoid outlet sphincters do the contracting We don't know which it is in man Personally, I think both things may be present in all species

Gyorgy: Why cannot you find it?

Knisely. Probably because no one has used the right way.

Mackay. There is work by [illegible] where with the use of digita [illegible] pressure. They suggest some [illegible]

Gyorgy They suggest, or did they find it?

Mackay. No, they present evidence that there is a fall in right auricular pressure

Best Histamine does not cause any swelling of the liver in the human species, does it?

Knisely: Not so far as I know

Best It does in the dog certainly. The sphincter is contracted by histamine in the dog, but if you use the same test you get no response in the human being

Knisely There is no reason to assume that histamine would necessarily cause the outlet sphincters of man to close just because this substance is an effective stimulant initiating the closure of the outlet control mechanisms in dogs In large numbers of histologic sections of livers of human beings I have hunted to find places where sinusoid outlets open into central veins. One does not often find the exact opening of a sinusoid into a central vein It is much

commoner to find that the sinusoid ends in a cul-de-sac right beside the central vein. This might be because the pathologic section did not happen to run through the opening. Or it might be because the outlet valves closed and stayed closed during and after the death of the individual.

The above suggests a key set of tests, such as setting up good fused quartz rod illumination apparatus, sterilize it well, and study the structure and behavior of the parts of living transilluminated human liver lobules during laparotomy.

Bradley: You are placing the block proximal to the central vein?

Knisely: Right where the sinusoid joins the central vein.

Bradley. What do you mean by "valve"? Do you mean a shelf of tissue?

Knisely. Desach observed the site where several sinusoids came together. He picked up the biggest sphincters of this class.

Bradley. At the level of the central vein?

Knisely· Yes, where several sinusoids join together and then enter the central vein.

Best You might get those sphincters contracting without any great swelling of the liver.

Knisely. In rabbits in one kind of shock, *all* these sphincters shut up so tightly that at no place can blood from a sinusoid enter a central vein. There is no remaining patent opening to the central veins. The whole liver then swells a little bit. The fluid part of the blood goes out through the sinusoid walls and dilates the Dissé spaces and the thoracic duct as well, which swells. Then there is sudden ascites, and the animal dies within ten minutes.

Watson: What kind of shock?

Knisely: We have been calling it "reservoir retention shock." The stimulus is a crude extract from lung. What it has within it we don't know

Best Lots of histamine

Hartroft Of what is the annular sphincter composed? Smooth muscle?

Knisely: We don't know Histologists like to believe they can stain everything in tissue That is not so. They believe if something is made out of smooth muscle it could contract, and if not it could not contract There are things in anim[illegible] can contract which are not made of simple smooth musc[illegible]

REFERENCES

1. LEE, F. C.: On the lymph vessels of the liver *Carnegie Institute Contributions to Embryology* 15, *63 (1923).*

2 KNISELY, M. H, BLOCH, E H, and WARNER, L: Selective phagocytosis I Microscopic observations concerning the regulation of the blood flow through the liver and other organs and the mechanism and rate of phagocytic removal of particles from the blood Det Kongelige Danske Videnskabernes Selskab, Biologiske Skrifter, Bind IV, Nr 7 København, 1948 (p 39)

3 MALL, F P., On the origin of the lymphatics in the liver. *Bull Johns Hopkins Hosp* 12, *146 (1901).*

4 POPPER, H Über Drosselvorrichtungen an Lebervenen *Klin Wchnschr* 10, *2129 (1931)*

5 BLOOM, WM: The role of the lymphatics in the absorption of the bile pigment from the liver in early obstructive jaundice *Bull Johns Hopkins Hosp* 34, *316 (1923).*

6 MAYO, C, JR, and GREENE, C H Studies in metabolism of bile, role of lymphatics in early stages of development of obstructive jaundice *Am J. Physiol* 89, *280 (1929)*

7 GONZALEZ ODDONE, M V: Bilirubin, bromsulfalein, bile acids, alkaline phosphatase and cholesterol of thoracic duct lymph in experimental regurgitation jaundice *Proc Soc Exper Biol & Med* 63, *144 (1946)*

8 BAUM, H Über die Einmundung von Lymphgefassen in der Leber in das Pfortadersystem *Verhandl d anat Gesellsch 56 (Erg Bd) 97 (1922);* Nach der Tierart verschiedenes Verhalten der Lymphgefasse der Serosa der Leber und der Lunge *Anat. Anz* 67, *88 (1929)*; *Das Lymphgefasssystem des Rindes* Berlin Hirschwald, 1917

9 KNISELY, M H, BARKER, S, BLOCH, E H, LIPSCOMBE, A, WARNER, L, BROOKS, F, DRAGSTEDT, L R, SCHNEIDER, C L, LEQUIRE, V S, STONER, H B, and IRWIN, J A new separation of several sets of pathologic mechanisms which occur during different kinds of circulatory "shock," and single one of which acting maximally can alone, by itself, cause peripheral vascular failure and the death of the subject *Anat Rec* 106 (Am Assoc Anat) *43 (1950)*

10 THOMAS, W D, and ESSEX, H E Observations on the hepatic venous circulation with special reference to the sphincteric mechanism *Am J. Physiol* 158, *303 (1949)*

11 KROGH, A, and LINDHARD, J Measurements of the blood flow through the lungs of man *Skandinav Arch f Physiol* 27, *100 (1912)*

12. KROGH, A.: On the influence of the venous supply upon the output of the heart *Skandinav. Arch. f. Physiol.* 27, *126 (1912).*

13. KROGH, A : The regulation of the supply of blood to the right heart, with a description of a new circulation model. *Skandinav. Arch f. Physiol.* 27, *227 (1912)*

14. MCMICHAEL, J, and SHARPEY-SCHAEFER, E. P.: Action of intravenous digoxin in man *Quart. J Med.* 13, *123 (1944)*

SECTION IV

THE ESCAPE OF LIPID FROM FATTY CYSTS IN EXPERIMENTAL DIETARY CIRRHOSIS[1]

W. STANLEY HARTROFT[2]

Banting and Best Department of Medical Research, University of Toronto

PATHOLOGICAL CYSTS filled with fat (previously termed lipodiastaemata), which are formed in the livers of choline-deficient rats during the development of cirrhosis, have been reported by the author(1, 2). It was found that many of these cysts atrophied and were replaced by fibrous tissue which comprised the trabeculae characteristic of experimental dietary cirrhosis in rats. Cystic atrophy occurred during that period in which it was found the total amount of hepatic lipid decreased, this decrease may thus represent the sum total of fat lost from many individual cysts. Routes by which lipid leaves degenerating cysts were not demonstrated and form the subject of the present communication.

METHODS

Microsections of tissues taken from over 100 Wistar rats of both sexes which had been fed a low-choline diet (page 110) for periods ranging from 9 to 15 months were studied by a variety of methods. Frozen sections treated with Oil Red-O by the technique of R D. Lillie(3) were counterstained with hematoxylin prepared according to the method of G. Gomori(4) and with Light Green, in place of the conventional Mayer's hematoxylin This modification (introduced by W D Wilson in our laboratories, publication pending) affords excellent contrast between small drops of scarlet fat and cyan cytoplasm or green erythrocytes

The biliary trees of selected experimental and control animals were partially filled with a filtered, diluted colloidal suspension of carbon * This was introduced under very low pressure to avoid artefact via a fine plastic tube secured in the bile duct This pro-

[1] This work was supported by a grant from the National Cancer Institute of Canada
[2] The writer is greatly indebted to Dr J H Ridout for preparation of the diets and assistance in many aspects of the work Mr William Wilson gave expert [illegible]

DIET:			
Arachin	12%	Beef fat	10%
Gelatin	6%	Corn oil	2%
Casein	3%	Cod liver oil concentrate	0 015%
Fibrin	1%	Cystine	0 50%

Salts	5%
Celluflour	2%
Sucrose	57.5%
Vitamin powder	1%

The composition of the salts used in the above, per 100 gms. was

Calcium lactate	35 0 gms.
Calcium carbonate	5 06 gms.
$Ca(H_2PO_4)_2.H_2O$	14 60 gms.
K_2HPO_4	6.46 gms.
$NaH_2PO_4.H_2O$	18.76 gms.
NaCl	9.34 gms.
$MgSO_4$ (anhydrous)	7.18 gms
Fe citrate	3.20 gms.

and trace salts:

$MnSO_4.4H_2O$	0 33 gms
$ZnSO_4.7H_2O$	0.035 gms
$CuSO_4.3H_2O$	0 039 gms
KI	0 00039 gms.

The Vitamin powder per 1000 gms. of mixture contained:

Thiamin . . . 500 mgm., Riboflavin . . . 250 mgms,
Pyridoxine . . . 200 mgms, Calcium pantothenate . . 1 gm,
Nicotinic acid . . 1 gm., and powdered sugar . . . 997.05 gms.

The cod liver oil concentrate used (C L.O. conc.) contained 50,000 I U. of Vit. D and 200,000 I.U. of Vit. A per gram. (Supplied by Messrs. Ayerst, McKenna and Harrison, Montreal).

All photomicrographs, unless otherwise specifically stated, are of microsections prepared from the tissues of rats fed low-choline diets for periods ranging from 9 to 15 months.

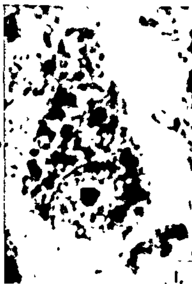
[illegible] hepatic [illegible]

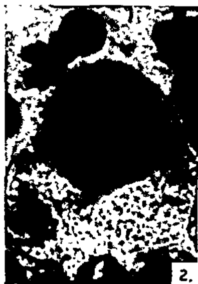
x 2200.

FIGURE 2. Two spherules of fat, one very large and one smaller are shown *in the* cytoplasm of a single hepatic cell, the nucleus of which (upper left) is in mitosis Frozen section of a weanling rat fed a low-choline diet for 8 days Oil Red-O, Light Green and Hematoxylin Oil-immersion — x 2200.

FIGURE 3 Two spherules of fat from adjoining hepatic cells are in the process of fusing to form a single pool of extracellular lipid (a fatty cyst). Frozen section of liver stained with Nile Blue Sulphate x 800 (Note the difference in magnification between this photograph and the preceding).

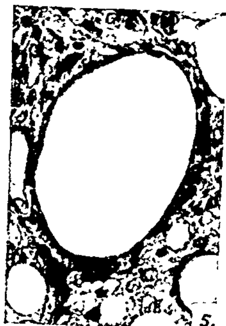
FIGURE 4 An appearance similar to that shown in Figure 3 taken from a paraffin section of liver This shows the "hour-glass" shape of the wall of a recently formed fatty cyst Such forms are transitory and soon assume circular outlines. *Hematoxylin* and eosin. x 800


1.


2.


3.


4.


5.


6.


7.


8.

duced complete injection of all bile radicles and adjacent canaliculi Thick (100 micra) sections of these livers were cleared in benzyl and benzyl benzoate following dehydration in isopropyl alcohol A limited three-dimensional visualization of portions of the biliary tree in both control and experimental animals was thus obtained. Frozen sections stained to demonstrate fat were also prepared from the injected livers.

Paraffin sections of all organs were routinely stained with hematoxylin and eosin, and in selected cases by a variety of special methods to demonstrate fibrous and elastic tissue, hemosiderin, hemofuchsin, acid-fast pigment, and ceroid (Oil Red-O applied to the paraffin sections). Most of the observations to be reported were made with the oil-immersion objectives of the standard microscope. When frozen sections were employed for this type of study, those not thicker than 4 to 5 micra proved satisfactory.

RESULTS

Observations indicated that the contents of fatty cysts could escape by at least two routes. These consisted of the biliary canaliculi and the hepatic sinusoids. Each of these pathways will be considered separately following a brief resumé of the formation and structure of the cysts.

Stainable fat initially appears in the cytoplasm of liver cells as small droplets which coalesce into one or two large spherules if



FIGURE 6 Several stages in the atrophy of fatty cysts which are becoming surrounded by fibrous tissue are illustrated These atrophic cysts may resemble abnormal bile ducts at this stage Azo carmine, Anilin Blue and Orange G x 600

FIGURE [illegible] number [illegible] area N[illegible] in and [illegible] G x 50

FIGURE 8 A low-power view of a paraffin section (x 50) of liver from a rat which has been fed a low-choline diet for several months and then transferred to a diet with choline added, for 12 days All *intracellular* fat has disappeared under the [illegible] ...t in fatty cysts [illegible] importance of [illegible] liver and also [illegible] ...orm The inset [illegible] of intracellular

the amount of lipid in the liver increases (Figures 1 and 2). Several spherules from adjacent cells may similarly coalesce to form a large pool of fat (Figures 3, 4 and *A* of Color Plate I), which, previously *intra*cellular, has now become *extra*cellular and fills the lumen of a newly formed cyst (Figure 5). Its wall is formed by the conjoined parent cells from which the fat escaped to enter into the central pool. Rupture of the limiting membranes of neighboring cells which had become distended by their contained fatty spherules, marks the transformation of the lipid from an intracellular to extracellular position. Evidence for this has been presented in detail by the author elsewhere(1, 2). Many fatty cysts formed in this manner subsequently atrophy (Figure 6), and become replaced by fibrous tissue which condenses into trabeculae. The nonportal distribution of the latter was first emphasized by L. L. Ashburn, K. M. Endicott, F. S. Daft, and R. D. Lillie(5) and later confirmed by L. E. Glynn, H. P. Himsworth, and O. Lindan(6) and by P. Gyorgy and H. Goldblatt(7). Around the trabeculae, fatty cysts may be seen in every stage of either formation or fibrotic degeneration (Figure 7). If choline or its precursors are restored to the basal diet after the extracellular transformation of some of the hepatic fat, only that lipid which is still intracellular is readily mobilized within a few days by the lipotropic factors which have been added to the diet (Figure 8). The escape of the extracellular fat from cysts which atrophy during the development of cirrhotic lesions in rats *maintained* on a choline-deficient ration will now be considered in detail

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FIGURE A A large fatty cyst in the liver of a rat fed the low-choline diet for 11 months is surrounded by the cyst wall composed of several cells, the nuclei of which are shown The lumen is completely filled with scarlet lipid Frozen section stained with Oil Red-O, Light Green and Hematoxylin. x 800

FIGURE B A chain of red fat droplets are entering a bile canaliculus which communicates with the lumen of a fatty cyst which has been partially depleted of its lipid That which remains is in the form of a crescent clinging to the inner surface of the wall Frozen section of the liver of a rat fed the low-choline diet for 11 months Oil Red-O, Hematoxylin and Light Green x 800

FIGURE C A [illegible]
[illegible]
green erythocytes x 800

FIGURE D. A large vein (probably an hepatic radicle) in the liver of a choline-deficient rat is completely filled with scarlet lipid in which erythrocytes are embedded The black material is ink which has been injected into the biliary tree. Frozen section stained with Oil Red O and Hematoxylin x 500

A **B**





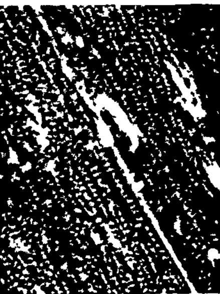




C

COLOR PLATE I

E



F





G



H

COLOR PLATE II

THE BILIARY PATH

Fat contained within a cyst may escape into bile canaliculi which open into its lumen and pass between cells which form its wall (Figures 9, 10 and *B* of Color Plate I). These communications are probably established when limiting membranes of cells separated by canaliculi are ruptured at the instant of formation of the cyst Fat droplets, apparently making their way toward portal triads, are found in bile canaliculi in mid-zones of lobules (Figures 11 and 12). Further evidence of communications between cysts and the biliary tree was found in sections of livers injected *via* the hepatic duct. In the frozen sections, a mixture of fat and ink is present not only in many of the bile ducts (Figure 13), but also in some of the cysts (Figure 14). In many fibrotic regions, bile canaliculi have persisted (Figure 15) although the parenchyma has disappeared, these are probably precursors of the new bile ducts which eventually form in the fibrous trabeculae. Cysts and bile ducts, both containing injected ink, can also be demonstrated in the paraffin sections (Figure 16). The normal pattern of the biliary tree in thick cleared slices of injected livers is shown in Figure 17. In similar prepara-

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[illegible]

Green and Hematoxylin x 800

[illegible]

inset, x 800

[illegible]

diet for 10 months Oil Red-O, Light Green and Hematoxylin, x 1200

FIGURE H A large fatty cyst in the liver of a chronic alcoholic patient coming to autopsy The cyst wall is clearly shown The lumen is completely filled with scarlet fat At the site indicated by the arrow (right), note a small spur of fat which appears to be [illegible] two of the cells of the cyst w[illegible] [illegible]reen and Hematoxylin x 600 [illegible] Boyd of the Department o[illegible]

tions of livers which contain fatty cysts, the latter appear as small ink-filled sacs (Figure 18) which surround the fibrous trabeculae.

If bile in canaliculi which communicate with cysts flows toward the latter rather than away, cystic distension rather than drainage could result. In livers of some animals studied, cysts of very great diameter were sometimes found. Ink injected into the bile duct failed to enter these giant sacs, although the media could be seen in canaliculi immediately adjacent (Figures 19 and 20). These special cysts may represent abnormal variants of the usual fatty cysts. Stainable lipid could not be demonstrated in frozen sections of the giant cysts. If their contents consist of a watery suspension of fat it is possible that a mixture of this type might be more easily lost during the preparation of the section than would be a mass of undiluted lipid compressed within the lumen of the type of fatty cyst usually encountered Further studies of the pathogenesis of these giant cysts are under progress and will be reported in the near future.

THE SINUSOIDAL PATH

The thinned wall of a distended fatty cyst may be so closely applied to the lining of an adjacent sinusoid that it is impossible to resolve the single tenuous partition so formed into its two constituent parts (cyst wall and sinusoidal wall (Figure 21)). Drops and even larger masses of fat have been found both in sinusoids and in radicles of the hepatic vein (Figures 22 to 27; *C* and *D* of Color

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FIGURE 9 At the positions of three, five and six o'clock on the wall of the fatty cyst in the upper portion of the photomicrograph, small droplets of fat are escaping through three bile canaliculi which penetrate between the cells forming the wall Frozen section of liver stained with Oil Red-O, Light Green and Hematoxylin x 800

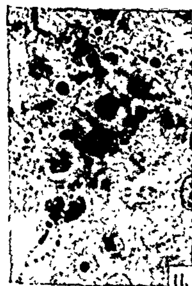
FIGURE 10 At the positions of ten, two and six o'clock on the wall of the large fatty cyst in the center-right portion of the field, fat droplets may be seen entering bile canaliculi Frozen section of liver stained with Oil Red-O, Light Green and Hematoxylin x 800

... from centro-
... and
... seen
...nally
...rozen
entered bile canaliculi in the manner depicted ...
section stained with Oil Red-O, Light Green and Hematoxylin x 800

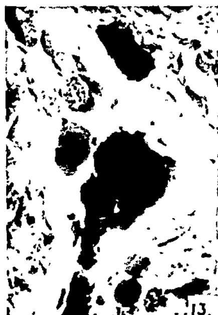
FIGURE 12 This field selected from the mid-zonal region of a liver lobule (see ... small droplets of fat
... stain-
... Oil


9.


10.


11.


12.


13.


14.


15.


16.

Plate I). In Figure 22 drops of fat may be seen which appear to have escaped from a nearby cyst into a sinusoid containing a few erythrocytes. Blood cells are sometimes found in the lumina of cysts only partially filled with lipid (Figures 23, 24 and *C* of Color Plate I). This suggests that when some of the fat in a cyst has been drained into a sinusoid, the space so created within the cystic lumen could be refilled by red cells. Such an occurrence would be a manifestation of relative pressures within cyst and sinusoid. Large plugs of stainable fat in sinusoids are shown in relation to cysts from which the fat may have escaped (Figures 25 and 26). Mixtures of lipid and erythrocytes in sinusoids can also be demonstrated (Figure 27) Figure *D* of Color Plate I) illustrates a vein, lying in a fibrous trabecula and completely filled with fat in which red cells are embedded As the relations of sinusoids and bile canaliculi to liver cells are similar, it is possible that the communications between cysts and blood vessels may become established by a process analagous to that already described for bile passages

CHANGES IN THE FORM OF CYSTIC LIPID AS IT ESCAPES

As fat escapes from a cyst, it undergoes a change in shape The lipid no longer forms an evenly rounded mass which completely fills the cystic lumen. The fat contracts to a reniform, crescentic or doughnut-shaped mass which clings to the lining of the lumen In microsections, the form in which the remaining lipid appears in

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he photomicrographs comprising this plate and that following are of sections of ers from rats injected with ink via a plastic tube inserted in the bile duct (see ext)

FIGURE 13 In this frozen section, both ink (black) and fat (grey) are present and mixed in a bile duct This illustrates that stainable lipid enters the biliary system of choline-deficient rats Oil Red-O, Light Green and Hematoxylin x 800

FIGURE 14 A mixture of ink and fat are present in the bile duct crossing the lower portion of the field. In the upper portion, a fatty cyst contains a thin rim of injected ink which has seeped into the structure and lies between the wall and the contained lipid (grey) Similar preparation and magnification to that shown in preceding figure

FIGURE 15 The region illustrated is in the center of one of the fibrous trabeculae present in the cirrhotic livers The ink injected into the biliary system has rendered visible the fine network of persisting bile passages which are present, but ordinarily escape notice, in the fibrous tissue It is possible that these persistent canaliculi are the precursors of bile ducts which form in the trabeculae Similar preparation and magnification as shown in Figures 13 and 14

FIGURE 16 Injected bile passages and fatty cysts in and near a fibrous trabecula are shown in a paraffin section stained with Hematoxylin and eosin x 600

cysts partially depleted of their contents, will depend not only on the amount of fat left, but also on the plane of section If a crescentic mass of cystic fat (lower left portion of Figure 28) *is cut in* the planes represented by the lines, A, B and C, the resultant appearance in the sections will be that shown in portions A, B and C of the same figure. Technical artefact would not be likely to produce a line of cleavage which would divide a mass of fat in the crescentic manner, and such forms or those shown in B and C are therefore considered indicative of ante-mortem loss of fat from cysts. As the cyst walls are never closely applied to the concave aspects of the crescentic masses of remaining fat, it is likely that atrophy follows, rather than precedes or produces the removal of lipid. Empty cysts no longer perform the useful function of storing excess hepatic fat, and their atrophy and fibrotic replacement conforms to the generally acceded principles of pathologic alterations

EMBOLISM OF LIPID IN HEPATIC SINUSOIDS

Evidence will be presented that embolism of the fat, which enters hepatic sinusoids from ruptured cysts, occurs to the heart, lungs and kidneys.

HEART. Elongated droplets of fat have been demonstrated in the lumina of capillaries lying between cardiac muscle fibers of cirrhotic rats (Figure *E* of Color Plate II). These findings are associated with

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The photomicrographs comprising this plate and that preceding are of preparations of livers from rats injected with ink via a plastic tube inserted in the bile duct (see text).

FIGURE 17 The normal biliary pattern as visualized in a thick (100 micra) cleared slice of liver from an injected control animal Only the canaliculi immediately surrounding bile ducts have been injected x 20. The inset demonstrates the manner in which canaliculi drain into ducts x 80

FIGURE 18 Similar preparation to that shown in the preceding figure, but from a rat [illegible] photograph above and slightly to the [illegible] black Newly *formed* bile ducts in [illegible] e seen surrounding the parenchyma forming an annular pattern. Note the numerous ink-filled sacs surrounding the fibrous tissue x 20 One of these sacs which represents a fatty cyst, is shown in the inset Note the injected canaliculus connecting the sac to a bile duct x 80

FIGURE 19 Two giant cysts (see text) lined by flattened epithelium are shown in a paraffin section The adjoining bile duct is filled with ink, but none has reached the large cysts Hematoxylin and eosin x 200

FIGURE 20 The bile canaliculus partially surrounding this giant cyst (see text) is filled with ink, but there is no demonstrable communication between the cyst and the biliary tree Hematoxylin and eosin x 200.


17.


18.


19.


20.


S


S
21.


E
S
22.


FC
S
X
23.


24.

small myocardial infarcts (Figure 29) composed of necrotic and calcified muscle.

LUNG. Fat droplets within alveolar capillaries (Figures 31 and F of Color Plate II) and small amounts of intravascular ceroid (Figure 32) can be demonstrated in pulmonic microsections of cirrhotic rats. Some of the intravascular fat appears to enter alveolar spaces (Figure 31) where it is engulfed by phagocytes (Figure 33) The latter accumulate in large numbers in lymphoid tissue (Figure 34) which surrounds dilated, thin-walled bronchi filled with exudate (Figures 35 and 36). The macrophages may have interfered with the lymphatic drainage of the affected bronchi, rendering them susceptible to infection with consequent weakening of their walls This may be the pathogenesis of a typical bronchiectasis with bronchopneumonia (Figure 30) which is responsible for the death of a significant percentage of rats fed low-choline diets for periods of more than a year.

KIDNEY. Drops of fat (Figure 37) and of ceroid (Figure 38) can be demonstrated in small renal arteries and arterioles of many of the experimental animals. Glomerular loops (Figures 39 and G of Color Plate II) are found which contain masses of embolic lipid Small amounts of fat are also demonstrated in the space of Bowman (Figure 40). Intravascular injection of ink indicated that those capillary loops which are filled with fat are impervious to the injection-mass (Figure 41) In paraffin sections, focal necrosis of

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The photomicrographs comprising this plate and that following are of frozen sections of liver which have been stained with Oil Red-O, Hematoxylin and Light Green They all have been prepared from rats fed the low-choline diet for more than a year

FIGURE 21 The sinusoids, (S) indicated by arrows, and partially filled with blood cells (pale grey) may be followed around the periphery of the fatty cysts illustrated. These vessels are separated only by thin membranes (see text) from the lipid contained within the cysts x 800

FIGURE 22 Fat droplets (black) lie in a sinusoid (S) which contains a few pale grey blood cells (Er) These droplets appear to be escaping from the retort-shaped cyst in the upper portion of the field x 800

FIGURE 23 The fatty cyst (FC) in the upper left is almost completely depleted of lipid except for a small crescentic remnant (black) Erythrocytes which appear to originate from the sinusoid at the right of the cyst, have entered and partially filled the lumen. At X in the lower right portion of the field a few tiny fat droplets (black) are entering the lumen of the *sinusoid (S) which contains pale grey erythrocytes* x 500

[illegible] the frame in the [illegible] ure of lipid and [illegible] in Figure C of [illegible] cle also contains

some glomerular tufts (Figure 42) suggests that this may be the result of fatty obstruction of capillary loops. Lipid is also present in casts in the cortical tubules (Figure 43) and to an even greater extent in casts filling the tubules of the medulla (Figure 44) where it is frequently mixed with deposits of calcium salts. The supernatent fluid of centrifuged urine collected from some of the experimental animals has been devoid of demonstrable globules of fat. This may suggest that all lipid reaching the medullary tubules may be precipitated as casts, so that little is excreted. This is further supported by the large amounts of fat often found in these tubules.

Fat embolism suggested by the foregoing evidence is regarded as chronic, repeated and mild in nature as compared with the acute massive form seen in surgical fat embolism in man. Small showers of fat droplets appear to be intermittently released into the circulation of rats in which hepatic fatty cysts have formed. Other organs than heart, lungs and kidneys may also be involved, but evidence of this has not been found.

CEROID

Ceroid(8) was present in the fibrous trabeculae of livers of animals comprising the present series. As already noted small amounts of ceroid were also demonstrated within vessels of the lungs and kidneys It is suggested that the latter is of embolic

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The photomicrographs comprising this plate and that preceding are of frozen sections of liver which have been stained with Oil Red-O, Hematoxylin and Light Green. They have all been prepared from rats fed the low-choline diet for more than a year

FIGURE 25. A large plug of fat still connected with that which fills the cyst may be seen partially filling a sinusoid which appears to communicate with the cystic lumen which is incompletely depleted of its lipid. x 800

FIGURE 26 The fatty cyst (FC), a portion of which is included in the upper left part of the field, has ruptured into the sinusoid which runs to the lower right Nearly all the lipid has left this cyst and a large plug of fat is present in the lumen of the sinusoid A few erythrocytes (pale grey shadows) are visible in the cavity of the cyst. x 800

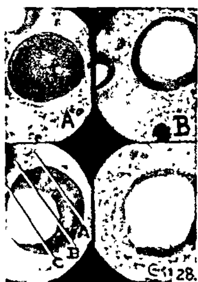
FIGURE 27 Fat (black) lies compressed within masses of red cells (grey) which fill the sinusoid passing from upper right to left center of the field. This vessel lies in the mid-portion of a liver lobule x 500.

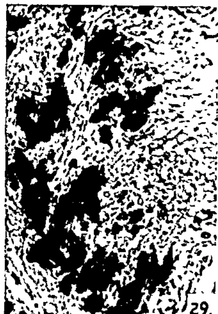
FIGURE 28 The lines, A, B and C indicate planes of section of the fatty cyst containing a crescentic mass of lipid (lower left), which would result in appearances in sections illustrated in the fields A, B and C It is unlikely that artefact could produce a line of cleavage through the mass of fat which would follow this shape (See text) Note blood cells mixed with the fat in some of the cysts Each field — x 500


25.

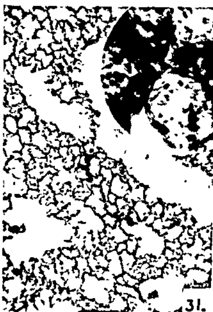

FC
26.


27.


A
B
A
B
C
28.


29.


30.


31.


32.

nature, originating from the liver. The presence of ceroid within the vascular system deserves further consideration.

In sections of livers which contain relatively little ceroid, many of the atrophic fatty cysts are filled with red cells and small amounts of hemosiderin, whereas both these features are less easily demonstrated in sections of liver which contain abundant ceroid (Figures 45 and 46). In frozen sections of the latter (stained with Oil Red-O, Hematoxylin and Light Green), collections of coarsely granular material may be found which resemble clumped red cells (Figures 47 and 48) that have lost their affinity for the green dye, although not sudanophilic. It is possible to show that the sudanophilia of ceroid varies greatly. The foregoing observations taken together suggested that under certain conditions ceroid might represent a product formed when red cells and lipid are intimately mixed in fatty cysts or sinusoids (Figures 23, 24 and *D* of Color Plate I). To test this hypothesis, washed red cells from animals fed the basal diet were mixed with cod liver oil *in vitro* and incubated for 5 days at 37° C with frequent agitation. The centrifuged sediment was washed in xylol and alcohol, smeared on albuminized glass slides or taken upon cigarette paper, fixed in formol-saline and stained with Oil Red-O and Light Green (Figure 49). Globules of coarsely granular orange pigment mixed with red cells which still retained an affinity for Light Green could be demonstrated in these preparations. This orange pigment also resembled ceroid in that it proved to be acid-fast by the Ziehl Nielson technique (Figure 51) and it reacted positively to Mallory's hemofuchsin stain as well as nega-

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FIGURE 29. Necrotic muscle fibers which have undergone fatty and calcareous degeneration are shown in this frozen section of cardiac muscle stained with Oil Red O, Hematoxylin and Light Green. These small infarcted areas may be related to small fat emboli present in the vessels, as illustrated in Figure *E* of Color Plate II. From a cirrhotic rat fed the low-choline diet for 11 months. x 400

FIGURE 30. The typical appearance on gross examination of the lung of a rat fed the low-choline diet for over one year and dying of bronchiectasis with bronchopneumonia. The characteristic mottled appearance of a liver which is slightly cirrhotic and losing lipid from fatty cysts is shown below.

FIGURE 31. The vessels which contain small black masses in the low-power view of this frozen section of lung from a rat choline-deficient for a year, are partially filled with embolic lipid. x 80. Such a vessel containing fat droplets is shown in the inset. x 600. Oil Red-O, Hematoxylin and Light Green. Also see Figure *F* of Color Plate II.

FIGURE 32. The capillary indicated by the arrow, C, in the alveolar septum in this paraffin section of lung is completely filled with a mass of sudanophilic ceroid. From the same animal as the preparation illustrated in the preceding figure. Oil Red O, Hematoxylin and Light Green. x 600

tively to the Prussian Blue test for hemosiderin. Tests concerning its *fluorescence will be conducted in the near future* When the sediment was not extracted with alcohol or xylol before staining, transition forms between erythrocytes which appeared normal and the orange pigment could be found (*Figure 50*), which resembled the forms considered suggestive of transition stages between red cells and ceroid in the frozen sections of livers. Further investigations are being performed, but it is suggested that red cells and lipid can react in some manner to produce either ceroid or a product resembling it. This would not rule out the possibility that other tissues or tissue-products may be involved. Such a concept explains the presence of break-down products of red cells (hemosiderin) in livers where ceroid is absent, and conversely, their absence in livers where ceroid is abundant, as well as the occurrence of ceroid emboli Finally, ceroid (*Figure 52*) and hemosiderin were found in upper abdominal lymph nodes which drain the liver.

THE POSSIBILITY OF CHRONIC FAT EMBOLISM IN ALCOHOLISM AND RELATED CONDITIONS IN MAN

Frozen sections of livers, lungs and kidneys obtained at autopsy from a series of chronic alcoholics are being examined for evidences of intravascular fat*. The series is not completed, but illustra-

* This material was generously placed at the disposal of the author by the kindness of Professor William Boyd of the Department of Pathology and Bacteriology, University of Toronto, Toronto, Canada

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FIGURE 33 Masses of stainable fat lie free and within macrophages in the alveolar lumen of the lung of a rat fed the low-choline diet for a year. Frozen section stained with Oil Red-O, Hematoxylin and Light Green x 500.

... filled ... lipid (black) have collected ... single macrophage ... the inset x 800 ... e and treated with the same stains

FIGURE 35 A large distended bronchus is almost completely filled with a mass of purulent exudate Paraffin section stained with Hematoxylin and eosin, from the same lung illustrated in the preceding two figures x 50

FIGURE 36 Giant distension of a bronchus, only a portion of which is included in the lower left, has occurred in another part of the lung illustrated in the preceding figures The smaller bronchus in the upper left contains exudate x 50 The inset illustrates the type of cellular reaction found around such bronchi. x 800 Paraffin section stained with Hematoxylin and eosin.


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34.


35.

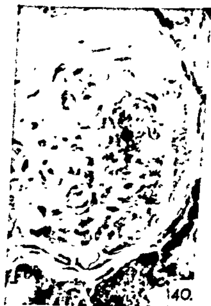

36.


37.



38.


39.


40.

tions are presented from one case (Female, age 35 yrs) Large fatty cysts in various stages of formation and atrophy were present in the sections of liver (Figures 53 and *H* of Color Plate II) Fat droplets were found in hepatic sinusoids (Figures 54 and 55) Small droplets of fat were found throughout the capillaries in many regions of the lung (Figure 56). The patient's terminal episode was characterized by bronchopneumonia It is possible that the presence of embolic fat in the lung may have constituted a predisposing factor to the occurrence of the fatal pulmonic lesion. The result of a more extensive survey of the organs of chronic alcoholic patients coming to autopsy will be reported in the near future

SUMMARY

1 In fatty and cirrhotic livers of rats fed low-choline diets for 9 to 15 months, droplets of stainable lipid from pathological fatty cysts (formerly termed "lipodiastaemata" by the author) which rupture, may enter either the biliary canaliculi or the hepatic sinusoids

2. Fat has been demonstrated within the lumina of cardiac, pulmonic and renal vessels of these animals The effect of the intravascular fat on the parenchyma in each case has been illustrated It is suggested that the embolic fat originates from the ruptured fatty cysts of the liver.

3 Ceroid pigment appears to be formed in livers of choline-deficient rats by the interaction of red cells and fat Evidence

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The photomicrographs which comprise this and the following plate are of sections of kidneys of rats fed the low-choline diet for periods ranging between 9 and 15 months

FIGURE 37 In this frozen section stained with Oil Red O Hematoxylin and Light Green, masses of stainable fat (dark grey to black) are mixed with red blood cells (pale grey) in the interlobular artery at the right and in the capillary loops of the glomerulus x 200

FIGURE 38 Sudanophilic ceroid (C) completely fills the lumen of an afferent arteriole leading to the glomerulus (Gl) shown indistinctly in the right hand portion of the field Only a portion of the glomerulus is included Paraffin section stained with Oil Red O, Hematoxylin and Light Green x 800

FIGURE 39 Nearly every capillary loop of the glomerulus which fills this field is filled with masses of stainable fat (black) Frozen section stained with Oil Red O Hematoxylin and Light Green x 600 See also figure G of Color Plate II

FIGURE 40 Small amounts of sudanophilic material (dark grey) lie in the space of Bowman (right portion of field) Some of the glomerular capillary loops also contain fat Frozen section stained with Oil Red-O, Hematoxylin and Light Green. x 600

obtained both *in vivo* and *in vitro* in support of this concept has been presented.

4. Chronic fat embolism may be a feature of the fatty cirrhosis seen in chronic alcoholism and related conditions in man.

Fremont-Smith: That was a wonderful presentation.

Best. Are there any questions on Dr Hartroft's presentation?

György: I have a few. It is too late to bring out all of them I congratulate you, Dr. Hartroft, on a wonderful presentation. It is really pioneer work, in giving us explanations for quite a number of old, unsolved problems. If my following questions are not quite in line with this praise, it is only because the time is too short to throw more bouquets to you which you deserve

As Dr. Hartroft has already stated, neither Dr. Goldblatt who has examined my rats maintained on a low protein-high fat diet nor Dr. Pappenheimer in his studies has found that ceroid formation could be prevented by the administration of tocopherol Pappenheimer, who was the first to point out the possible relation of ceroid to the pigment found in animals with tocopherol deficiency, con-

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The photomicrographs which comprise this and the preceding plate are of sections of kidneys of cirrhotic rats which had been fed the low-choline diet for periods ranging between 9 and 15 months

FIGURE 41 The vessels of the animal from which this preparation was made, were injected with India ink Note that although the capillary loops of some of the lobes of the glomerular tuft have been penetrated by the ink, those in which there are droplets of fat (pale grey) have failed to fill with the injected material Frozen section stained with Oil Red-O, Hematoxylin and Light Green x 400

FIGURE 42 Focal necrosis of one lobe of the glomerular tuft is present (NE) in ... the top half of the field The ... ing It is possible ... by embolic fat ... G x 250.

FIGURE 43 ... amounts of palely sudanophilic ... precipitated within medi... Oil Red-O, Hematoxylin and Light Green x 400

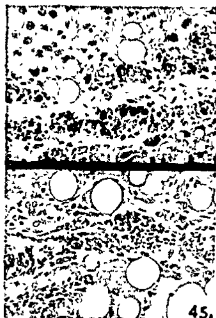
FIGURE 44 Nearly every tubule present in this low-power view of the medulla is plugged by masses of sudanophilic lipid (dark grey) which is undergoing calcification (black) x 150 Individual tubules are shown in greater detail in the inset (x 500). Frozen section stained with Oil Red-O, Hematoxylin and Light Green.


41.


42.

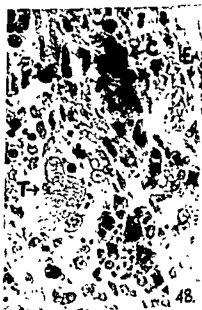

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48.

cluded in his paper published jointly with Victor(9) that tocopherol may delay the development of ceroid in experimental cirrhosis but will not completely suppress it. We are of the same opinion, based on our experimental observations. It should also be mentioned that Endicott(10) if my memory does not fail me, injected rats subcutaneously with cod liver oil and found "conversion" of this injected cod liver oil into ceroid *in situ*(10), apparently without the concomitant production of a hematoma and thus, without the interaction of red blood cells.

Further, cirrhosis may be produced in rats fed a tocopherol-free diet without the simultaneous production of ceroid, provided the diet is free or very low in fat(11).

It has always been our feeling that the absence of ceroid in human cirrhosis is due to the fact, which is quite understandable, that in general, men, including the cirrhotic patients, prefer alcohol to cod liver oil. Cod liver oil is the most important dietary factor in the production of ceroid. I have possibly misinterpreted you, but I got the impression that in your opinion red blood cells are somehow



FIGURE 46. The
with ceroid stain
positively reacting
preceding figure
with the same sta
Note small granu
Blue. Paraffin sections

FIGURE 47. A large mass of lipid in a cyst occupies the center of the field (fat appears black). Masses of ceroid at the right appear almost as dark. Below the cyst and slightly to th
(if at all) surlanc
which have lost tl
rat) stained with

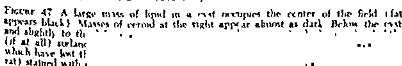


FIGURE 48. A
grey masses
fibrous tissue
at the right
of altered re
sudanophilia. x 800

converted to ceroid, whereas Mason, as well as our own group, are inclined to link ceroid with polymerized unsaturated fatty acids, such as are present in cod liver oil. Admittedly, protein may be attached to the polymerized fat but we have no proof for it that this *protein would come from red blood cells.*

According to Lilhe and his group(8) ceroid may be found not only in the liver, but also in the spleen, in phagocytic cells of lymph nodes, in the adrenal glands, bone marrow and the lungs. The question arises whether ceroid may be produced only in the liver, and reaches the organs through emboli and consecutive phagocytosis or may it originate in other organs as well. It seems that Dr. Hartroft *gives his preference to the liver as the sole organ of ceroid-*production.

I would like to ask two more questions: Regeneration of hepatic parenchyma often occurs in choline deficient rats, even after many months on the alipotropic diet. Such newly formed nodules are usually free from fat, although they develop in animals suffering from chronic choline deficiency. What is the explanation for the *absence of fat in these newly formed hepatic cells?*

FIGURE 49 Clumps of granular, sudanophilic material are shown in the upper and lower left portions, which were present in the sediment formed in vitro by a mix- ... cod liver oil incubated with ... urried ... nized ... d in ... ip of ... terial ... Light Green) All x 500

FIGURE 50 In the upper half, a fatty cyst containing a mixture of red cells (grey) and fat is present Some of the red cells appear to be "transition forms" of ceroid. (Frozen section of liver stained with Oil Red-O, Light Green and Hematoxylin x 500) In ... a mixture of cod liver oil (forming a crescentic mass) and red cell ... the sedi- ment (see ... ms" (This sedim ... and Light Green) x 400

FIGURE 51 ... of liver from a choline-de ... cid-fast. Ziehl Neilson stain ... perimental rat with cod li ... in the lower half x 200

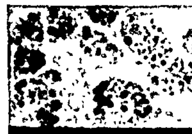
FIGURE 52 Sudanophilic ceroid in macrophages within an upper abdominal lymph node draining the liver of a choline-deficient rat, is shown in this photograph of a paraffin section stained with Oil Red-O and Hematoxylin x 400.

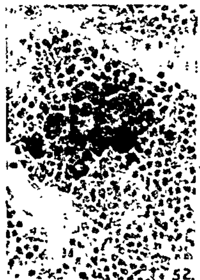





49.


50.


51.


52.

of ceroid, but only that under certain conditions, these cells may alter the physical chemistry of liver lipids so that their reactions resemble those of ceroid. The absence of hemosiderin deposits when ceroid is present in appreciable amounts in cirrhotic livers, and the presence of hemosiderin when ceroid is absent or scanty in amount, suggest that the formation of ceroid *in vivo* under these *conditions interferes* with the deposition of the usual products which result from the break down of red blood cells. If hematomata are not produced in experiments where ceroid followed the subcutaneous injection of cod liver oil, this may imply that some tissue element other than red blood cells can alter the physical chemistry of fat in a manner similar to that in which red cells appear to act. Further experiments would be necessary to supply the answers *to these questions.*

Handler Did you ever use red cell hemolyzates in the oil?

Hartroft: Only washed erythrocytes have been used in the experiments reported.

Watson: You think it is the hemoglobin or the red cell itself?

Hartroft: I can only speculate. The next question is concerned with the absence of ceroid in the cirrhotic livers of alcoholic patients.

Gyorgy: They don't consume cod liver oil That is the reason they don't have ceroid

Hartroft: It is possible that any liver oil, liver oil from rats, from humans, or from cod fish may under suitable conditions function as a precursor of ceroid. The absence of ceroid in the livers of chronic alcoholics might be due to the presence of adequate amounts of tocopherol in the diet of these patients.

Gyorgy Cod liver oil is far superior to any other fat. Other unsaturated fats are much milder ceroid producing materials.

Patek. I realize that this is a controversial subject. However, it should be mentioned that Pappenheimer and Victor(9) described a substance that closely resembled ceroid in human liver tissue.

Hartroft We have not been able to confirm these findings.

Popper: We saw a pigment of ceroid fluorescence in 3 or 4 human livers in a series of several hundred cases of cirrhosis.

Hartroft: Professor William Boyd of the Department of Pathology, University of Toronto, kindly allowed me to examine 40 cases of

alcoholic cirrhosis. Significant amounts of ceroid in these livers could not be found, but in many of these we found small deposits of hemosiderin pigment. Pappenheimer reported ceroid in organs other than liver, — the lungs and kidney. In the rat, ceroid may be found in hepatic and pulmonic macrophages and it is possible that these may be drained to the regional lymph nodes. If some of the ceroid globules are free within the circulatory system, they may be picked up in the spleen. The evidence I presented indicated the presence of ceroid in the lumina of renal vessels This suggests that perhaps it is unlikely that ceroid is formed in the kidney, but must be brought to it.

It was asked why so little stainable fat is found in young liver cells which form the nodular areas of parenchymal regeneration. By the time this stage in the development of cirrhosis is reached, the rats are always older and their growth rates correspondingly low. More methyl groups are then available for lipotropic purposes. Perhaps these nodules undergo only slight degrees of fatty change simply because at the time of their formation the animal is not so severely choline-deficient as in the earlier stages of the cirrhotic process.

Best. There should be some questions on ceroid

Handler: As to the last few remarks of Dr Hartroft, I don't think I like that particular explanation It does not seem particularly satisfactory. For one thing, if one uses regenerating liver from young rats, the regenerating liver becomes just as fatty as normal liver in the same time on choline-deficient diets If you start out with a rat which weighs 400 grams, place it on a choline deficient diet it takes a relatively short while to produce the usual fatty liver, and you have started with an animal whose main growth phase is over.

Best Nevertheless, in the regenerating liver the islands of new cells are not fatty

Handler I have no explanation

Hoffbauer Of the two pathways you discussed, which do you think is the more important in so far as the removal of fat is concerned?

Hartroft. Fat entering the circulation in the form of small drops would of course exert a harmful effect on the animal in the form of embolic phenomena, while fat entering the biliary system probably has little effect on the animal's general health

Best: There would not be enough to make a difference

Hartroft: Embolic fat appears to be very damaging, even in small amounts if these emboli are frequently repeated They are responsible for lesions of the kidney, lung and heart, as demonstrated in the slides. The embolic type of renal damage is superimposed on the lesions of the kidney produced more directly by choline deficiency(12).

Popper. Dr. Hartroft's paper brings up quite a few important aspects. I would like to ask whether the central nervous system, to which fat emboli very commonly go, was examined. Maybe these observations offer an explanation for the changes in the central nervous system observed in liver disease.

Dr. Ivy and Dr Nelson produced by dietary means in guinea pigs peculiar changes which were histologically studied by Dr. Paul B. Szanto in our laboratory. Parallel with marked fatty livers, vascular changes of arteriosclerotic character were ushered in by the appearance of lipophages in the intima of arteries Did you see, in the arteries of your rats, lipophages or other vascular changes? Such an observation would possibly suggest that fat embolism from the liver may be the cause of the vascular changes observed in our material.

Moschcowitz(13) recently presented excellent evidence for the development of new vessels in the trabeculae of the cirrhotic liver which process he called angiogenesis. According to your observations, some of these apparent vessels would actually be fat cysts.

Finally, I would like to suggest a possible tagging of this fat, actually a negative tagging. I believe the fat in these large cysts is free of vitamin A fluorescence or at least very low in it. On long standing the vitamin A may disappear from the fat. It would be interesting to investigate whether the fat in the emboli is free of vitamin A This would present fairly good circumstantial evidence that the emboli derive from the hepatic cysts and not from peripheral fat tissue If the fat of the emboli would be free of vitamin A fluorescence, it would be good evidence; if they would contain it, it would not prove anything

Best: It seems to me that these embolic phenomena are things to follow up. That offers a great many interesting possibilities I think Dr. Hartroft has already seen a lot in human alcoholic cirrhosis which had not been seen before.

Davies: These observations interest me very much. In African pathology we have been puzzled by the frequent association of

hyalinized glomeruli and patchy fibrosis in many other organs, with fatty liver in infancy, an association we were not able to explain. The kidneys of African children and young adults often resemble in this respect the kidneys of old men. I think we shall have to start looking into the possibility of such mechanisms as you have described being operative in these cases.

Best: Do these children's livers go on to the formation of fatty cysts?

Davies: I think they do, but we did not know what they were.

Knisely: Two points, sir: Many of the places which you showed were not very big and the fat droplets might squeeze out as they are going through the vessels. Just for fun, it might be possible to study these things during life by sticking a microscope in the living animal, no difficulty at all. That might let you see whether the fat droplets begin to stick to red cells. Some seemed to show fairly good fatty surfaces in terms of surface tension, some being miscible.

This leads to one more point. It might not be at all difficult to draw a sample of rat blood, centrifuge, and in terms of volumetric chemistry not get very much of it. But that amount might be vastly more than enough to run some tests under the microscope to see if you could make ceroid with the rat's own fat. It does not sound very hard.

Watson: What would be the matter with the perirenal fat from the rat?

Knisely: I was thinking of the very same fat already shown. Let us get the fat circulating, put it through the circulatory system.

Watson: You may not be able to get enough that way.

Hartroft: The preparations illustrating ceroid-like substance, which had been produced *in vitro*, were all treated in the same manner as paraffin sections in that the substance was passed through absolute alcohol and xylol to extract any unaltered oil. In addition, extractions were performed with ether. There was only one exception to this procedure, as was noted when the slides were presented. This was the preparation of ceroid-like substance, produced *in vitro*, used to illustrate transition forms between the insoluble form of cod liver oil (ceroid-like) and unaltered oil. In this instance the alcohol, xylol and ether solutions were omitted to preserve the transition forms of the oil which would otherwise have dissolved[1] in the fat-solvents.

Best: What is the possibility that there is never anything like ceroid, that it is all made by the sectioning, putting it through the staining procedure? I am not suggesting this too seriously. It would be quite a joke if there was not any ceroid, that it was all made by the staining procedures which you go through.

Knisely: Let us not believe that.

Handler: How long had these animals, with huge cysts and emboli, been on the choline deficient diets?

Hartroft: Fatty cysts were found in livers of rats, initially weighing 125 grams, which had been fed our standard choline-deficient diet for 35 days. Cysts have not been noted in any of our material prepared from animals of the same initial weight which had been fed this diet for periods less than five weeks.

Best: These pictures of the large globules, what stage were they?

Hartroft: The material illustrated was obtained from animals fed the choline-deficient diet for much longer periods.

Best: Fourteen months?

Hartroft: A year or more. In reply to an earlier comment by Dr. Popper, I should state that we have prepared frozen sections from the brains of a few rats fed low-choline diets for periods of 6 months to one year. Stainable fat was not demonstrated in the cerebral vessels, nor were any lesions found that might be considered the result of embolic occlusion of arteries or arterioles. The percentage of the total circulating blood which passes through the brain is perhaps less for a rat than for human subjects in which, under suitable conditions, the brain is well known to be frequently subject to embolic occlusion of vessels

Best: Dr Hartroft made a point that the fat in these large cysts might get out by the lymphatics or bile canaliculi, or via the sinusoids. Dr. Tarver and I have been comparing notes, and obviously Dr. Hartroft appreciated this possibility also, that the fat might have got out by more normal mechanisms. It might have been utilized in the liver or turned into ketone bodies, or whatever the fate might be normally. It is quite likely that that does not happen very readily to the fat in these abnormal cysts; nevertheless, the possibility has to be kept in mind

I thought we should emphasize a little bit more and it came out particularly, I felt, in Dr Schiff's slides, that these fatty cysts don't

respond well to the administration of lipotropic agents, or more fundamental than that, don't respond well to starvation or undernutrition which takes the fat out of the more normal liver cells very rapidly.

It should perhaps be emphasized in our minutes that clinicians who are doing liver biopsies might strike an area of these fatty cysts after they have used certain therapeutic procedures and still find a lot of fat there. This is what you might expect to find. The conclusions might be quite different depending on the area sampled. Does anyone want to comment on that?

Stetten: I would be interested, Dr. Hartroft, in learning something of the nature of this fat. I think the following experiment could be done on some of your one-year choline-deficient rats. Give them an adequate supplement of choline so as to eliminate the bulk of the intracellular fat, as I understand it this does not rapidly mobilize the fat in your cysts. Then take the liver out and mince it and do chemical analyses on the fat and learn whether it is highly unsaturated, as Dr Gyorgy thinks it may be, or whether it is mostly triglyceride.

Hartroft: I think that is an excellent suggestion

Stetten From the slides it would appear that 10 percent or more of the liver wet weight might well be fat in some of your preparations

Hartroft. I have subjected the lipid in these liver sections to the usual histochemical tests As you probably appreciate, these are not as informative and accurate as we might like I have been unable to detect any difference histochemically between intracellular hepatic fat and lipid contained in the cysts

Best They appear bigger than a kidney glomerulus, perhaps you can put a pipet into one and suck the fat out.

Shorr I wonder, Dr Best, whether it might not be possible to remove the fat from the cysts by a procedure that has been employed to remove colloid from fresh sections of the thyroid For example, if thin sections of thyroid are prepared and floated on a Ringer solution, the colloid drops out of the acini and becomes available for analytical procedures It should be possible to treat liver sections in the same way

Hartroft In our experience very little fat falls out of sections of well-fixed tissues

5. ASHBURN, L. L, ENDICOTT, K. M., DAFT, F. S, and LILLIE, R. D.: Nonportal distribution of trabeculae in dietary cirrhosis of rats and guinea-pigs *Am J. Path.* 23, 159 (1947).

6. GLYNN, L. E HIMSWORTH, H P., and LINDAN, O: Experimental production and development of diffuse hepatic fibrosis ("portal cirrhosis"). *Brit. J. Exper. Path* 29, 1 (1948).

7. GYORGY, P., and GOLDBLATT, H.: Further observations on the production and prevention of dietary hepatic injury in rats *J. Exper. Med.* 89, 245 (1949).

8 LILLIE, R D, *et al.* Histogenesis and repair of hepatic cirrhosis in rats produced on low protein diets and preventable with choline. *Pub. Health Rep.* 57, 502 (1942).

9. VICTOR, J., and PAPPENHEIMER, A. M.. Influence of choline, cystine, and of *a*-tocopherol upon occurrence of ceroid pigment in dietary cirrhosis of rats. *J Exper. Med* 82, 375 (1945)

10. ENDICOTT, K. M.: Similarity of acid-fast pigment ceroid and oxidized unsaturated fat. *Arch. Path.* 37, 49 (1944).

11 ENDICOTT, K. M, DAFT, F. S, and SEBRELL, W. H · Dietary cirrhosis without ceroid in rats *Proc Soc. Exper Biol. & Med.* 57, 330 (1944).

12. HARTROFT, W. S: Some histological aspects of renal and hepatic acholinopathy. *Liver Injury.* Hoffbauer, F. W., Editor Trans Sixth Conf. New York: Josiah Macy, Jr. Foundation, 1947 (p. 41).

13 MOSCHCOWITZ, E · *Laennec's cirrhosis· its histogenesis,* with special reference to role of angiogenesis *Arch Path.* 45, 187 (1948).

SECTION V

KWASHIORKOR

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I WOULD like to start by saying what great pleasure it gives me to be invited to join one of these celebrated conferences, whose proceedings in the past I have followed with great interest. It is a pity in many ways that you are listening to me this morning rather than to my senior colleague, Dr. H. C. Trowell, who has worked so many years on this disease and whose knowledge is so much greater than my own. We have worked together very closely these last five years and I am not sure now who first thought up many of the things I am going to present to you this morning.

I would make one further point: I am a servant of the Uganda Government and I am speaking here by permission of the Director of Medical Services, Uganda, who has kindly given me a general permit to speak on scientific subjects as long as I do not reflect, in any way, on the policies of the department. Anything I say is, therefore, entirely my own.

This curiously named disease is by no means a new disease. I think there is clear evidence in the literature that it has occurred from time to time in Europe(1) and indeed an epidemic occurred during the recent siege of Budapest(2). In the tropics it was for a long time completely overlooked because of the importance attached to parasitic disease. If you go back into the tropical literature, however, you do find clear evidence that they recognized there were existing states of malnutrition, not precisely defined and extremely difficult to treat(3) often complicated by parasitic disease. These conditions became more notable when the vitamin era set in and diets devised to cure vitamin deficiency diseases were found to be ineffective in these conditions. The stage was well set in the middle twenties of the century when in East Africa Procter(4) described the disease in children and the similar condition of "Bouffissure d'Annam" was attracting attention in the Far East(5, 6). In 1933 and in 1935 Dr. Cecily Williams(7, 8), described the disease in very considerable detail as it is seen in West Africa. Her

reports were followed by further descriptions of the same disease from the Belgian Congo, Central and South America and from other parts of the world, as can be seen from the extensive bibliography attached to Dr. Trowell's recent paper(9). The disease was characterized as occurring in children about the time of weaning, manifested by edema, depigmentation of the skin with peeling, particularly in the flexures; anemia, curious changes in the hair, and with steatorrhoea. This is the first picture they drew and it is an extremely accurate one. It was noted at autopsy that the liver was fatty(7, 10) and that there was thymic atrophy and intestinal atrophy(11). Most unfortunately the disease was miscalled pellagra(12) and was regarded merely as pellagra occurring in children(13). For some years, despite the fight waged against these views by Williams(8, 14) this view reigned supreme. Generally speaking little interest was taken in the disease in the tropics. This was not surprising for if it was pellagrous in origin, well, pellagra was being carefully investigated in this country and it was thought reasonable to wait till you gave us a remedy and then we would clear up this disease magically. The chemists kindly provided us with nicotinic acid, it was tried out and though it certainly had some action on the skin lesions(15), the children still died and indeed a sure way of killing them was to give them vitamins (16, 17). So there we were, back where we started from in the early thirties Then the news of the Gillmans' liver biopsy studies in South Africa came through and Dr. Trowell invited me to join him in a further attack on the problems of this disease.

Being heavily burdened with routine work and teaching and with very limited facilities, our attack had to be one of great breadth without very much depth and we have gone from one attractive aspect of the disease to another without ever going very deeply. We first investigated the fatty liver, almost the one fixed point at that time, using the liver biopsy technique while we also studied the livers of those coming to autopsy. Dr. Trowell was entirely [illegible] work in

Not till we reviewed the material later did I know [illegible] the material had come. We had imagined we would find fat constantly present in considerable amount in the liver and in the first few cases fat was present in considerable amount, and it looked too easy, but then absolutely typical cases came down in which there was little or no fat in the liver(18). The problem became complicated once more and it was not till we constructed a table

of the relationship of age to the presence of fat in the liver could we see some way ahead. (See Table I.) The vast majority of the cases, at least of the acute phases, fall between the ages of one and four years, but there are many cases in older children and in adults. Dividing our material into groups corresponding to the ages of the patients we found that, in young children, there was an intensely fatty liver with little fibrosis and little cellular accumulation in the portal triads, but as they got older, there was less and less fat and more and more fibrosis and cellular collections. Then in the 5-10 age group this was still more apparent, the fat being less and less conspicuous and in many typical cases was absent, but as the fat disappeared so did the fibrosis become more apparent. The same progress was noted in the teen-agers and in adults.

PATHOLOGY OF LIVER AND PANCREAS IN KWASHIORKOR. (50 Cases.)

TABLE I

Ages	LIVER			PANCREAS	
	Fat Infiltration	Cells in Portal Triads	Fibrosis	Atrophy	Fibrosis
0.5 - 5 years	+ to ++++	+++	± to +	+++	± to +
5 - 10 years	+ to ± to 0	++	+	+	± to +
10 - 20 years	0 to ±	+	+	+	+
Over 20, Locals	± to +	++	++	++	+ to ++
Over 20, Immigrants	+++	+++	++	+++	+ to ++

We could divide the latter into two groups, first of all the local residents who just happened to have a short period of malnutrition or a mild period of malnutrition. In these the fat in the liver was present in relatively small amounts but the fibrosis was much more marked than we had seen in children and there was considerably more cellular accumulation. In the second group were immigrants who had been forced in the immediate post-war period, to walk vast distances to work in Uganda and were not transported, as they are nowadays, by bus. On the way many suffered from severe malnutrition and they presented much the same picture as in young children very marked fatty infiltration but again more fibrosis and more cell collections.

Our problem, therefore, was that the fat in the liver did not correspond at all with the clinical picture of the disease There might or might not be fat present. It was clear to us there was a cycle going on of fat infiltration and fat removal from the liver while the clinical condition continued. Therefore we started to look for changes elsewhere. Our attention was turned to the pancreas and we were surprised to find an extreme degree of atrophy of the pancreas. We thought that this did begin to make sense, especially when we found that the pancreatic atrophy long preceded the fatty infiltr[illegible] the li[illegible]

We had been trying choline and other lipotropic agents with little success in the treatment of these children. Dr. Trowell decided to abandon these attempts and give large quantities of protein. He gave them considerable amounts of steak. This diet was tried on several children – and this was followed by what we took to be an outbreak of mumps in the ward affecting these same children. The parotid glands became very swollen, warm and tender, and we thought it strange that no secondary cases of mumps arose. Then we tumbled to it. We fed steak to a number of children and every time we gave such a child enough meat – out came the parotid. So we saw we were getting to something really interesting, for if this happened to the parotid, what happened to the pancreas? On palpation the pancreas was tender, maybe it was warm, but it was certainly tender; about two to three days later the stools, which had, up to then, been full of undigested food and which more or less mirrored what went in the mouth, suddenly cleared up, reverted to normal and the child began to improve very rapidly.

So we began to get a picture forming in our minds of a disease characterized by a failure of the enzyme secreting glands to manufacture digestive enzymes. We turned our attention to the small intestine which we had been inclined to overlook previously and there was the same atrophy of the enzyme secreting glands

Then just as I was leaving Uganda quite another line of argument, that I shall come to later, struck us and we wondered if the lachrymal gland was also involved. So we have a conception of a disease in which the enzyme-secreting glands atrophy and fail to function, the brunt of the damage falling on the pancreas. In consequence, presumably of the pancreatic defect, and I think these children are about in the position of a depancreatized animal, a fatty liver develops and the consequences of prolonged fat infiltration ensue.

Best: They don't get any diabetes?

Davies: They are not diabetic. We might continue from here by discussing the clinical manifestations of the disease I am not a clinician and would refer you to the clinical descriptions(9, 11, 15, 19a-d). In brief, an African child does quite well on the breast for about five or six months, but he usually stays on the breast for about two or three years with occasional breast feedings up to the age of six years(7) This prolonged period of breast feeding is general all over Africa and indeed among primitive peoples elsewhere How it has come about, I don't know, possibly as a contraceptive measure, perhaps to reduce the incidence of gastroenteritis, but certainly they stay on the breast for a long period, and certainly by one year they are falling behind There is failure to grow and gain weight, they develop depigmentation of the skin, changes in the hair, a black dermatosis which may peel especially in the flexures

Handler: Is there any solid food supplement at all at this time?

Davies: Not as I understand(20), then or later on they go on to an adult diet consisting almost entirely of carbohydrates and, to make it palatable, it is watered down to a gruel, thus further reducing its nutritive value(7)

Abdon Do the mothers show signs of deficiency?

Davies Yes The children become edematous and there is a tendency to ulceration and infection, especially in the peeling areas The edema disguises the falling weight and the picture is not one of marasmus In fact, though the fat on the limbs may be reduced, there is if anything, an increase of the trunk fat In my experience, at autopsy one often cuts through a layer of fat a centimeter or more thick

Much the same picture is seen in adults with the addition of a crazy-pavement dermatosis the skin looking as if covered with plaques of black enamel Following the administration of nicotinic acid there may be active peeling of the hyperpigmented skin, but few other favorable changes

In those who recover from the acute stage stunting is frequent, the usual picture being that of a stunted child with the typical African pot belly and one can predict the presence of much undigested food in the feces

Perhaps the most striking changes are in the hair, the nice tightly coiled wool mat of the healthy African child is altered to a coarse rank straight hair of a dusty brown or even red, or even, in severe cases, a flaxen color. The more the weights of African children fall below normal the more likely they are to have these hair changes(20).

When it comes to the etiology of this condition we are now satisfied that parasites play no direct part. You find cases with malaria, ancylostomiasis, and other parasites, but you find other cases with no infection or infestation whatsoever. We think the cause of the condition is a dietetic deficiency, especially a protein deficiency There is extreme lowering of the plasma proteins, particularly the albumin fraction falling, while the globulin fractions may be elevated especially if there is an infection present(21). When we come to the pathology – there is atrophy of the pancreas and other enzyme-secreting glands; Waterlow(1) in the West Indies showed that there was atrophy of the striated muscle, fatty liver infiltration, and later fibrosis, and pancreatic atrophy.

Hanger: Do these children lachrymate normally when they weep?

Davies: They can certainly cry but I would not say what the quality of the tears was.

In our cases in Uganda we see the same lesions as described by Waterlow(1) perhaps, however, varying in severity. The pancreas is grossly atrophied with marked atrophy of the enzyme-secreting cells, the zymogenic granules have completely disappeared and the cells are reduced to nuclei with a thin rim of cytoplasm around them(18, 22) (Figures 1, 2). In fact, in severe cases the pancreatic cells contain no secretory substance whatsoever. There is no apparent change in the ductal epithelium. This atrophic picture is monotonously similar in these children There are no changes of note in the blood vessels. Later there is an increasing amount of fibrous tissue present and the pancreas may become quite sclerotic. I think there is possibly a true enlargement of the islets. These always appear enlarged when the acinar tissue atrophies, but I think that in these children there may be actual hypertrophy. However, no pointed study of this has been made. In all our cases we saw no cystic dilatation, no retention of secretion, nothing, in fact, resembling Anderson-Farber disease or cystic fibrosis of the pancreas. I cannot agree with those who suggest there is a similarity(23, 24, 25, 26). To me the picture is entirely

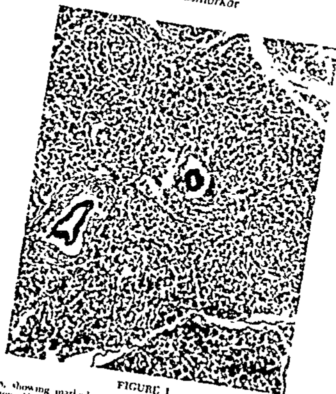


FIGURE 1

Pancreas, showing marked acinar cell atrophy from a child with marked fatty liver infiltration (H & E)

I should be surprised if there were any similarity between a condition in which secretion is lacking, none being produced to be retained and block the ducts, and one in which precisely this effect is produced.

In the parotid there is again (Figure 3) the same atrophy of the acinar cells without ductal or blood vessel changes and without cellular infiltrate, the same changes as seen in the pancreas (18, 27).

In the liver the fat infiltration is always initially peripheral in the liver lobule, periportal and not centrolobular. (Figures 4, 5) It is said possibly correctly in certain cases, that you cannot make any decision upon this point unless you inject the liver blood vessels to establish the proper architectural arrangement, but we are fortunate in some ways in working in the tropics and in this field we

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When it comes to the etiology of this condition we are now satisfied that parasites play no direct part. You find cases with malaria, ancylostomiasis, and other parasites, but you find other cases with no infection or infestation whatsoever. We think the cause of the condition is a dietetic deficiency, especially a protein deficiency. There is extreme lowering of the plasma proteins, particularly the albumin fraction falling, while the globulin fractions may be elevated especially if there is an infection present(21). When we come to the pathology – there is atrophy of the pancreas and other enzyme-secreting glands; Waterlow(1) in the West Indies showed that there was atrophy of the striated muscle, fatty liver infiltration, and later fibrosis, and pancreatic atrophy.

Hanger. Do these children lachrymate normally when they weep?

Davies: They can certainly cry but I would not say what the quality of the tears was.

In our cases in Uganda we see the same lesions as described by Waterlow(1) perhaps, however, varying in severity. The pancreas is grossly atrophied with marked atrophy of the enzyme-secreting cells, the zymogenic granules have completely disappeared and the cells are reduced to nuclei with a thin rim of cytoplasm around them(18, 22) (Figures 1 [illegible] the pancreatic cells contain no secreto[illegible] is no apparent change in the d[illegible] picture is monotonously similar in these children There are no changes of note in the blood vessels Later there is an increasing amount of fibrous tissue present and the pancreas may become quite sclerotic. I think there is possibly a true enlargement of the islets. These always appear enlarged when the acinar tissue atrophies, but I think that in these children there may be actual hypertrophy· however, no pointed study of this has been made In all our cases we saw no cystic dilatation, no retention of secretion, nothing, in fact, resembling Anderson-Farber disease or cystic fibrosis of the pancreas. I cannot agree with those who suggest there is a similarity(23, 24, 25, 26) To me the picture is entirely different and

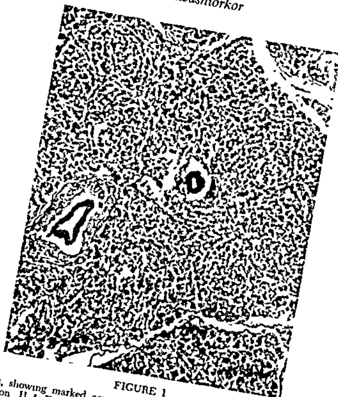


FIGURE 1

Pancreas, showing marked acinar cell atrophy from a child with marked fatty liver infiltration H & E

I should be surprised if there were any similarity between a condition in which secretion is lacking, none being produced to be retained and block the ducts, and one in which precisely this effect is produced

In the parotid there is again (Figure 3) the same atrophy of the acinar cells without ductal or blood vessel changes and without cellular infiltrate, the same changes as seen in the pancreas (18, 27)

In the liver the fat infiltration is always initially peripheral in the liver lobule, periportal and not centrolobular. (Figures 4, 5). It is said, possibly correctly in certain cases, that you cannot make any decision upon this point unless you inject the liver blood vessels to establish the proper architectural arrangement, but we are fortunate in some ways in working in the tropics and in this field we



FIGURE 2

Pancreas, showing atrophy and absence of ductal epithelial changes H & E

have one particular advantage, namely, that the liver of the malarious African is injected with malaria pigment. Studies of malaria pigment, as mentioned by Dr. Knisely, show that in the acute stages it is scattered diffusely throughout the liver lobule. It then passes to the Kupffer cells and from there it passes into the peripheral areas of the lobule and in the periportal spaces. Therefore African livers are already satisfactorily injected as further injection studies have confirmed In Figure 5 you see that the larger fat globules are well away from the central vein and the greater accumulations are in the periphery of the lobule, though there is often a sparing of the cells in the lamina limitans around the portal triad(28). Just inside the lamina there is a deposition of small droplets of fat that increase rapidly in size and as they are increasing peripherally small droplets appear nearer and nearer to the center of the lobule, the process going on steadily till the whole lobule is full of fat. In a



FIGURE 3

Parotid gland from a child with kwashiorkor, showing secretory cell atrophy H & E

really severe case one can see no single liver cells, save perhaps in the lamina limitans, which is not grossly distended with fat When these cases are treated with meat or milk, or very slowly if you give them choline, or even without treatment at all, the fat disappears first from the centrolobular region This is a slow retreat, the droplets getting smaller and smaller in size and finally disappearing, but from the peripheral region last of all With the fat accumulation there is an infiltration of round cells in the portal triads and in some circumstances in the sinusoids As Waterlow showed and as I can confirm, there is a thickening of the reticulum around these peripheral liver cells and they become surrounded by a thick wall of reticulum fibers which apparently transforms into collagenous fibrous tissue I must say that down in the center of the lobule, anywhere, in fact, away from the periphery, I have not seen

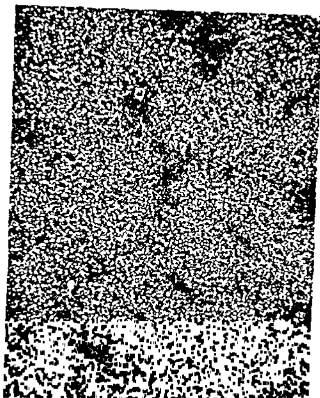


FIGURE 4

Liver from a kwashiorkor child showing marked fatty infiltration, malarious pigmentation and cellular infiltration H & E

any fibroblastic proliferation. The fibrosis that comes about seems to come by thickening of the reticulum tissue around the peripheral cells and extending down along the sinusoids.

When you give meat or milk to one of these children so that fat is being mobilized rapidly from the liver, and biopsies are done at frequent intervals, one of the first changes we have noted is a flooding of the liver sinusoids by lymphocytes. They come in such large numbers that the first time I saw it I thought the child had developed lymphatic leukemia. The cells lie in columns along the sinusoids and stay there as long as fat is disappearing from the liver. Later they disappear from the sinusoids but remain in the portal triads.

Knisely: During this process are the lymphocytes in the circulating blood increased?



FIGURE 5

Liver showing greater accumulation of fat globules to the periphery of the lobule with many cells in the sinusoids and in the triad. Malaria pigment also present. H & E

Davies. They don't appear to be particularly increased. I don't know what they are doing. It recalls to my mind the old observation that there are lipases in lymphocytes(29, 30, 31). I have a strong suspicion that there is a connection between rapid fat removal from the liver and the presence of these lymphocytes.

Watson: Is there no evidence of the development of phagocytes or lipophages from the lymphocytes?

Davies: I have not noticed them.

Knisely. Do you see any evidence of the lymphocytes being phagocytized by big phagocytes of the liver? That could be a very rapid process.

Davies: I want to say that our own observations, which are only superficial, seem to indicate that they are independent. In adults

exactly the same changes are seen, but it appears that there is considerably more fibrosis and more cellular infiltration in the portal triads (Figure 6) than one finds in children. We interpret this, rightly or wrongly, as being due to the fact that every African child goes through an attack of this disease in infancy, at least one attack This leaves behind, as a hallmark, fibrosis and cellular collections in the portal triads. These are seen almost universally in Africans (32, 18, 17) and indeed I have not yet seen an African liver I would class as histologically normal even by relatively lenient European standards, let alone by the strict criteria laid down by Elias(28). I would venture to say that there is hardly such a thing as a normal African liver in East Africa.

This fatty infiltration and associated fibrosis can lead rapidly to a stage that I think is frankly a Laennec cirrhosis (Figure 7) The

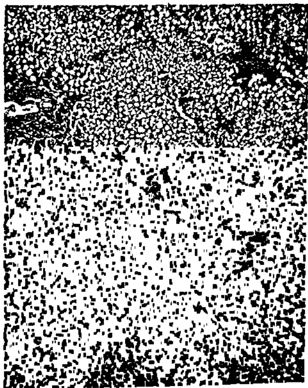


FIGURE 6

Liver from an adult with kwashiorkor Compared with the child there is more cellular collections in the triads and more fibrosis There is much fat present H & E

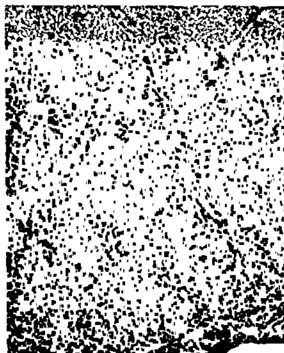


FIGURE 7

Liver from an adult with kwashiorkor showing a degree of change resembling a Laennec cirrhosis H & E

fatty liver in children can apparently give rise to a true monolobular cirrhosis

Gyorgy. Which lobule?

Davies. Monolobular, diffusely throughout the liver, identical in the distribution of the fibrosis with the pattern of biliary cirrhosis except that there is not such a tremendous accumulation of inflammatory cells around the lobules, no bile retention or bile duct hyperplasia The liver is about normal size, very granular, with very fine

similar to that described here by Dr Hartroft I don't think you can really call it a necrosis I don't like the term for this condition in which you see a coalescence of the fat-laden cells in a particular

area of the lobule, (Dr. Best illustrated it in his book(33)), in which we see a whole group of cells suddenly seeming to lose their *limiting membranes* There is a local accumulation of fat and it is through these areas that portal tract and central vein establish connections. I described this, very poorly, in my original paper(18) and called *it* a "*Himsworth* Lesion" through an entire misunderstanding of some work done by Dr. Himsworth. We thought the accumulation took place by a "sectorial necrosis" of the lobule I *don't like this term* "*necrosis*" but I don't know what to call it.

Since apparently every *African* goes through a stage of fat infiltration of the liver with the development of a certain amount of patchy stellate fibrosis and cellular accumulations, it follows that one cannot, *in the African liver, make the clear distinction made by* Himsworth(34) between a post-infiltrative fibrosis, as I prefer to call it, and a post-necrotic scarring. If necrosis does occur in an *African liver it does so in a liver bearing the scars of a fatty infiltra*tion, in the form of a patchy monolobular fibrosis. When you add to this schistosomiasis, hepatomatous change and a variety of other *changes you can well understand why the African liver* has been aptly referred to as a "histological nightmare"(35). The mixed nature of the cirrhosis of Africans was pointed out by Vint(32) *We have not yet gone deeply into the cirrhoses* in Uganda, we have only been trying to follow the fatty liver pattern.

I would now like to make a few general comments on the liver in kwashiorkor. As I said, the liver is intensely fatty in the acute *stages, but later the fat disappears, with treatment, or without treat*ment, so long as the patient survives Frequently in the late stages, ... abdominal fat which is often marked in the acute

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in South Africa(36, 17). The lar... of iron ...es

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of kwashiorkor and it is in these cases we also see an increasing amount of fibrosis of the liver. In some there is marked pancreatic fibrosis and little liver fibrosis; in others the picture is reversed, in some the fibrosis is roughly equivalent in each organ In some cases there is such a cellular infiltrate as to constitute a pancreatitis; more usually there is no such infiltrate. In the kidneys we have been puzzled by the frequency of hyalinization of the glomeruli(38). In the initial stages of this there is a metaplasia of the capsular epithelium to a more cubical form Associated with this is a hyalinization beginning round the base of the tuft and in the pericapsular

zed tuft with an outer

ten coalesce In many

para-amyloid material

in these sites, but this is not seen in all cases The adrenals also show changes but we have hardly begun as yet to study them So far as we have been able to determine, these changes consist of an increase in size of the cortex with an absence of pigment in the juxta-medullary zone so that on cross section there is a richly yellow cortex surrounding a white central region. On section the medulla is found to be fibrosed and in some cases there is a lot of nerve tissue present but we have made no pointed study of this We have not yet, because of our limited facilities, begun to study the other organs but I will say a few words about the heart in this disease

In the descriptions of the disease known as "Bouffisure d'Annam" about twenty-five years ago, and it appears to be the same as kwashiorkor, Normet(39) drew attention to the presence of a heart lesion consisting of mural thrombi in the ventricles associated with an hydropic degeneration of the myocardium. This was attributed to beri-beri, but it did not respond to the treatment of beri-beri It was therefore regarded as "atypical beri-beri," one of those queer diseases of the tropics We in Uganda have not been impressed by the presence of mural thrombi in the hearts of kwashiorkor cases, though we have been looking for them carefully However, they do show hydropic degeneration of myocardial fibers in the ventricles

However, there does occur a form of heart disease in Africa in which there is severe hydropic degeneration of the myocardium irresponsive to thiamin and with mural thrombi and patches of fibrosis in the cardiac chambers, particularly in the ventricles(40, 41, 42) It was recognized by Dr. Evan Bedford in African troops in the Middle East during the last war, and sometime later, in entire

ignorance of his work, I also recognized it in Uganda. At first I called the condition "endocardial fibrosis." Now I prefer to call it "endo-myocardial necrosis" because in the acute stages there is a hydrop[illegible] ers, a water [illegible] beri-beri. [illegible] iamin at any stage and in true cardiac beri-beri the response is so rapid as to be of diagnostic value(43). The hydropic vacuolation, if severe, leads to a peripheral striatal break up of the remaining cytoplasm of the fiber with ultimate death of the fiber. I do not want to take up much time on this disease as it will be fully described elsewhere, but, in brief, large areas of the subendocardial myocardium die slowly, with minimum cellular infiltrate and without blood vessel changes, the endocardium frequently degenerates with the deposition on it of thrombotic material which may or may not become infected and the necrosed areas are organized into firm white fibrous tissue At autopsy there is often a firm white plaque of fibrous tissue in one or more of the cardiac chambers, most characteristically in the ventricles and especially in the left ventricle. There may be a mass of adherent thrombus partially organized, or even completely surrounded by a white ring of fibrous tissue surrounding a dark mass of amorphous tissue. Histologically there is a thickened endocardium and a granulation tissue layer in the region of the old endocardium with broken-up elastic fibers. Underneath this there is more fibrous tissue with atrophied myocardial fibers with large nuclei in them or fibers showing the hydropic vacuolation The process is usually confined to the inner one-third of the myocardium or the papillary muscles only may be involved. The blood vessels are normal.

Dr. Towell has felt, and I agree, right from the start of our recognition of this disease, that there might be some connection between kwashiorkor and endomyocardial necrosis. Obviously there are other factors operative that we do not appreciate at this time I have not heard of any description of such a condition as this in experimental animals and I mention it here because of this group's wide experience of animal malnutrition, and because we are very anxious to know if anyone has met anything like it.

Patek: I wonder whether the [illegible] al changes encountered in vitamin E deficiency might be [illegible] ance to these.

Davies: I [illegible] d that two [illegible] horities think this might be due to v[illegible] ficiency. [illegible] n un [illegible] determine

any other etiologic factors and it might well be vitamin E deficiency. These people do not show any constant association with any other condition save malnutrition. One curious point is the absence of cardiac hypertrophy since these cases go on for several years with congestive failure and endocardial sclerosis. Save in circumstances where there is an obvious cause for hypertrophy, aortic syphilis or hypertension, these hearts are always atrophic The cardiac failure may go on for several years, with no response to therapy save the prevention of waterlogging, often with a persistently high diastolic pressure. The relationship of this disease to endocardial sclerosis with cardiac hypertrophy as seen in this country(44) is obscure. We suspect that in our cases there is some factor lacking which by its lack causes the fibers to atrophy and also prevents hypertrophy.

Shorr: May I ask what the incidence of hypertension is in this population?

Davies Could we defer that?

Stetten. We encountered once by chance a cardiac lesion which was much more acute than this and probably has no relation to it, but I would like to raise the following question What is the major fatty acid in the diet of these individuals? I make this inquiry because of the following observations In a study designed to determine the relative fattiness of choline-deficient livers, rats were raised on pure fats We observed that lauric acid, that is the 12 carbon fatty acid, when fed at relatively high levels uniformly produced death in young rats in from 4 to 6 days after starting the diet This occurred if no choline or methionine was added The death was due to congestive failure The hearts showed fragmentation of the myocardium, considerable edema of the myocardial fibers and in addition, an extensive invasion of the myocardium by polymorphnuclear leukocytes The hearts were sterile as far as we could tell, no bacteria could be grown This could be completely prevented by the addition of normal dietary amounts of either methionine or choline but could not be prevented by inositol

In this connection, I wonder if coconut is eaten in this area?

Davies· No

Best What are the fatty acids?

Davies I don't know

Best They would have to be given in large amounts if it is to be comparable to the animal experiments

Stetten: Yes, we gave 35 percent. Myristic and decanoic acids did not do it. Lauric acid killed every rat.

Davies: One or two other features of this disease: I saw it chiefly in malnourished people or, at least, in people who were at one time malnourished. I could not, dealing with autopsy material, define any other factors. Dr. Bedford's cases were well-nourished African soldiers, though they too had probably had past periods of malnutrition. However, when the illness arose, they were on an army diet. I think he was impressed in his cases with a history of severe physical exertion or the autopsy findings of hypoplastic aortae. We know, however, that many Africans have hypoplastic aortae(45). We wondered if a prolonged past period of dietary deficiency plus some strain factor might account for this disease. But Dr. Bedford tells me that he has now seen the disease in Europeans who have lived in the tropics. It ought to be reported frequently from West Africa as the bulk of Dr. Bedford's cases were West Africans.

Knisely: How soon after severe exertion did they die?

Davies: I cannot tell.

Knisely: As individual men while working?

Davies: No, no.

Knisely: In two weeks?

Davies: Much longer than that

György: Could it not be like Dr. Best and Dr. Hartroft's choline experiment, temporary deficiency in early life and then just not complete restitution to the normal?

Davies. That was in our mind.

Best: It would be very interesting to look into the fatty acid This observation of Dr. Stetten is an amazing one. It is apparently very clear cut.

Stetten: I would like to see it reproduced We of course suspected our supply of lauric acid. We obtained lauric acid from various sources, redistilled it and yet found that it always killed the experimental animal.

Gyorgy: Lauric acid is one of the most toxic fatty acids for almost all bacteria.

Stetten: It is an ingredient of many insecticides as we learned later.

Davies: To return to our initial discussion now. A survey of the geographic distribution of kwashiorkor reveals that it is now known to occur to a greater or lesser extent all over Africa It is seen south of the desert belt, in Egypt, in the West Indies, in Central and South America, in India, Assam, Indo-China, Annam, and, I believe, Dr. Gyogy has a report from Indonesia So we may say that it is a disease seen, to a greater or lesser extent, all over the tropics(9).

What of the etiology of this disease and what do we think is happening in these cases? In considering the etiology I would begin by pointing out that, because of the universality of liver damage we may assume that all the mothers have the marks of past malnutrition and possibly have some continuing defect. Add to this various tribal taboos so that women do not eat chicken, eggs or milk, it is not surprising that the children are born underweight, and it is my belief, and I think that of other workers in Africa, that not only are they underweight, but they are actually somatically immature. It is easy to suspect this, but the proof of it would be a frightful task. At any rate we think that they are born with a poor biochemical and somatic background, because all the mothers are nutritionally damaged. They do moderately well on the breast at first, then they begin to fall behind and then the catastrophe of weaning hits them, and about this time one observes the onset of kwashiorkor This is well recognized by the Africans themselves, as witnessed by the multiplicity of tribal names in different languages for the disease The name "kwashiorkor" means the "red boy" in a West African tongue, but it is known in Southern Uganda as "musanvu" These names reflect roughly two aspects of the disease, the striking changes in the hair and skin, or the fact that it is "the disease of the displaced child" The Africans well recognize that it occurs when a second child comes along and pushes the first off the breast of the mother to be weaned directly onto an adult diet This is when the disease ensues rapidly The children appear to be on a very low protein, very low fat and very high carbohydrate diet The mother endeavours to push the latter into them in large amounts so that the total caloric intake may be quite high, isocaloric or even hypercaloric At the same time, the diet contains a great deal of roughage(21) The vitamin content of the diets in Uganda appears to be more or less adequate, and we think that when a vitamin deficiency condition does appear in this dis-

Hanger: That may have some bearing on the paucity of fibrotic lesions in the livers of these patients

Davies: They seldom show a leukocytosis; there is almost a minimal in response to infection.

Fremont-Smith: Is it the custom to break off the breast feeding? In some countries they go back and forth and get a good deal of milk while the other child is nursing. Is anything of that happening or is there a taboo against that?

Davies: I don't think it is a taboo, but I don't think it is so often done. But other women may give the child an occasional breast feed.

Fremont-Smith: So for all intents and purposes the arrival of a child is the end of breast feeding for the other children.

Shorr: Have any studies been done on bottle feeding?

Davies. I don't know about bottle feeding per se but children reared under European observation in orphanages do not show this disease.

Shorr You are forced to conclude there may be something else missing in milk other than the total amount of protein, which you say is only minimally reduced?

Davies. I don't think protein itself, sir, is the key to this I think there are some other factors.

Madden. Has any study of possible trace element deficiency been made like manganese and zinc?

Davies. I think we have got trace deficiencies in another type of circumstance. I don't think they play any major role in kwashiorkor.

Mackay: When you say the vitamin intake is adequate, what do you mean?

Davies: They show no deficiency by and large, except possibly nicotinic acid deficiency.

Mackay. Is that based upon food analysis?

Davies: No

Mackay· The other point was, when you give vitamins, then they die?

Davies Yes often

Handler. Isn't that an ancient finding that rats on a multiple

deficiency diet fare lots better than when they were maintained in any single deficiency state alone?

Best. Isn't that related to a low caloric intake?

Knisely: It isn't by any chance that the bacteria which are in and around the various parts of the body are existing on a low vitamin diet and that you suddenly feed up the bacteria when you give vitamins to the patients?

Davies: I don't know. These people seem to settle down to a low metabolic plane. If you give vitamins you unbalance the whole economy. The Gillmans saw in South Africa the specific lesion improve remarkably; the hyper-pigmented skin will peel off, and the lesion will clear up like magic but the child dies two or three days later.

Kinsell: What was the nature of the death?

Davies. Not specific hypervitaminosis. As far as I understand they just fade out, pass into stupor, and die in coma(47).

MacKay: As to the details of the diet, you only mentioned plantains. What else do they have?

Davies. That is about all, literally about all. The diet on the whole consisting of boiled up plantains, perhaps a little sweet potato, very little meat, a certain amount of green leafy food.

Best. Do they ever keep plantains dry like meal or corn?

Davies: No, it grows the whole year round. I would not lay any stress on the particular type of carbohydrate in the etiology of this disorder, either banana, millet or maize can be responsible. You have got to have a high carbohydrate diet and low protein diet.

Madden: Millet or maize are likely to be unbalanced in amino composition. I wonder about the plantain.

Davies. It has been analyzed(52).

Handler: I am not quite certain that actual millet or maize are seriously unbalanced. Single proteins such as zein may be so. We are not yet ready to ascribe pellagra simply to an unbalanced amino acid mixture in corn or millet.

Watson. I would like to ask a question about the fat in the liver. I was very much interested in the relatively small amounts of fat in the livers that had the most fibrosis, as I understood it, later on in the life of the individual. I presume that that is not adequately explained by any increase in lipotropic factors in the diet?



FIGURE 8

Autopsy liver specimen (14-b) showing extensive scarring with little cellular reaction. (For details, see text.) x 190

Knisely: One possible explanation if the patients have demonstrable anemia, they may also *consistently* have low blood volume.

Davies: Many of them don't have severe anemia in the cirrhotic state

Knisely: They may still have low blood volume.

Davies. That is a possibility. That has been looked into Our cirrhotics vary, on the whole in the fatty liver cases the livers are enlarged but the liver shrinks as fibrosis develops. We also see a tremendous amount of subacute hepatitis and hepatic necrosis, etiology quite unknown, although we suspect strongly that much of it is nutritional. We get little outbreaks of acute yellow atrophy with practically 100 percent mortality but those show very small livers.

Gyorgy: Not infectious hepatitis?

Davies: I don't know I think we see it I believe we see serum jaundice

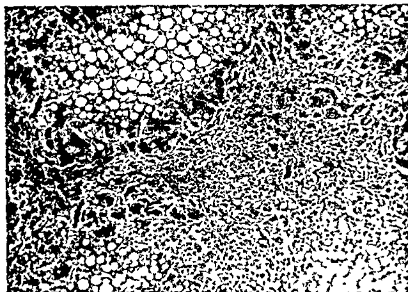


FIGURE 9

Autopsy liver specimen (15-b) showing fading away of liver cells at the periphery of the lobules and inflammatory infiltration of portal triads x 190

Hanger. It strikes me the livers you have demonstrated in these Africans have a relatively anaplastic mesenchymal component You don't get the scarring and you don't get portal hypertension and therefore you don't get the large scarred livers You find small contracted livers, as these cells degenerate, but acute necrosis is absent Is it possible that you have a racial characteristic and that factor may be the key to your picture?

Davies· I would suspect it is not racial because the Negro is characterized by ability to form fibrous tissue

Hanger. He does in this country

Davies· In Africa keloids are extremely common.

Gyorgy: In contrast to kwashiorkor, in children dying from hepatic disease in Jamaica, the pancreas is often found normal For instance, in spite of very severe hepatic changes (Figure 10) the pancreas may be free from pathological manifestations (Figure 11)



FIGURE 10

Autopsy liver specimen (6-c) showing peripheral collapse of framework and inflammatory reaction x 190

Davies. I agree, this is not a kwashiorkor pancreas. Might I ask whether they noticed any familial incidence of this disease? I ask that because I heard in connection with the infantile cirrhosis in India that once one child has developed it every subsequent child born to that mother will develop it.

Gyorgy. That familiar incidence is found mainly in high Brahman castes According to information received from India, weekly purgation with castor oil is in these families a common custom, applied even in young infants Perhaps the toxic castor oil, possibly combined with nutritional factor, may play an etiological role in the development of this familiar cirrhosis in India

Best. Do they get ceroid?

Gyogy I suspect, but I cannot prove, that they should have ceroid. As a matter of fact, according to the report of Dr. Blankart from Indonesia the most severe cirrhosis is seen on some islands in children and in adults who have in their diet shark oil as a major source of fat Shark oil with its highly unsaturated fatty acids should

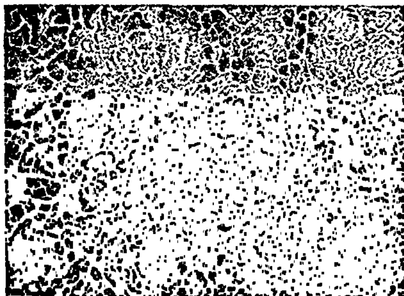


FIGURE 11

Autopsy specimen (6-d) normal pancreas from same case as shown in Figure 10 x 100

lead to ceroid. When the Indonesian mail arrives, we should get some interesting liver specimens

In Jamaica there is widespread malnutrition This manifests itself in children in normal or almost normal total caloric intake with a proportionately very low intake of protein, especially animal protein I give you a glaring example, obtained through the courtesy of Dr K Rhodes (Jamaica) who made not only the direct measurements of food intake per meal but also the necessary calculations A 5-year-old child weighing 21 pounds (!) and 37½" tall, consumed weekly 538 grams corn meal, 538 grams brown sugar, 113 grams condensed milk, 600 grams of green bananas, 453 grams of yellow yams, 56 grams of white bread (and bush-tea) The total caloric intake, 774 calories per day, is fairly satisfactory, at least with regard to the reduced existing body weight However, the daily protein intake, 11 7 grams, is certainly far under par, especially considering the fact that only slightly above 10% of the total protein intake is represented by animal protein of high biological value

In Jamaica, apparently in contrast to Africa, cirrhosis, at least according to our hurried observations, is relatively rare in adults. On the other hand, dietary neuropathies are very common in adults, such as "burning feet," retrobulbar neuritis, deafness, loss of vibratory sense, of superficial reflexes, etc. I would like to raise the question whether the difference in the appearance of these nutritional diseases may be linked with total caloric intake. Infants and children seem to have in Jamaica, just as in Africa, sufficient total caloric intake In contrast, the adults are underfed and may even starve in Jamaica. It is possible that starvation protects the liver and may adversely influence the nervous system. Reverse would then be true for children with their satisfactory total caloric intake and low protein intake.

How far toxic factors may intervene both in the production of hepatic injury or neuropathies, requires further studies. At present the low protein intake appears to be the main underlying dietary factor.

In this connection it should be pointed out that kwashiorkor, malignant malnutrition, fatty liver and similar nutritional diseases occurring over a large tropical belt of the world, extending from Indo-China over Indonesia, India, Africa, the West Indies, Central and parts of South America are very reminiscent if not identical with the so-called mehlnährschaden described first about 50 years ago by Czerny in Germany in children fed for a protracted period of time food mixtures containing only or chiefly flour or other forms of carbohydrate About 30 years ago Schick, Wagner and Priesel described atrophy of pancreas, salivary glands, fatty diarrhea — *without cystic fibrosis of the pancreas* — in so-called celiac syndrome, here again in analogy to the findings in kwashiorkor as presented by Dr Davies.

Protein deficiency and its consequences are a world-wide problem It affects without exaggeration perhaps 1500 million people or more. The implications, even from point of view of world politics, are obvious From the scientific angle the problem deserves our closest attention.

Davies: I think the literature is very respectable but it has been overlooked (see Jackson(55)) It would appear that Bloch(56) first noted pancreatic cell atrophy in malnourished children although previously Arraga and Vinas(57) had noted pancreatic changes in malnourished infants, possibly mostly fibrocystic disease But all

this work and that of subsequent workers appears to have been ignored because no distinction was drawn between marasmus and the kwashiorkor type of malnutrition which appears to be the same as the condition known to European pediatricians as "mehlnährschaden" or "starch or flour dystrophy"(48). It seems general experience that marked fatty liver infiltration and pancreatic atrophy are rarely seen in marasmus, or gastroenteritis or "pedatrophy" or "athrepsia" but are the predominant lesions in mehlnahrschaden and kwashiorkor Upon this distinction the earlier work appears to have foundered and so was lost for a time.

Best: I think Dr de Maeyer is going out from Belgium to the Congo

de Maeyer: I want to know why you see the deposition of pigment in the livers in Uganda. In the Belgian Congo no deposit of pigment is seen in the livers of the natives

Gyorgy: We don't see it in Jamaica.

Davies: We wondered if it was related to the fact that the iron content of maize, which is consumed to an enormous extent in South Africa, is much higher than banana. It has about 14 times the amount of iron in it as the banana But I understand what iron there is in the banana is much more readily assimilable than the iron in maize. If work recently done by the Cleveland group(58) is correct, we ought to see it in the liver We don't see it and we cannot explain it The explanation that it is due to tropical parasite infestation is quite incorrect

de Maeyer In the Congo, the diet is mostly mango and cassava and a little palm oil

Popper: Did you see, in addition to what you call monolobular cirrhosis, the wide scars with regularly spaced vessels seen in postnecrotic cirrhosis? In one of Dr Gyorgy's specimens such a picture can be noted

Davies: We see a tremendous amount of postnecrotic scarring, really gross postnecrotic scarring We see a tremendous amount of Laennec's cirrhosis, a lot of intermediate types, when both are present. We also see, but very rarely, what I think is a true monolobular cirrhosis I don't want to imply that we see that commonly I just saw two in several hundred cases I think fatty liver can go on to a true monolobular cirrhosis

Fremont-Smith: Could I say a word at that point? I think if what we know about the psychology of human development and social development applies in Africa, and I can see no reason from anything we do know about it to suggest that it does not, then your statement is of profound importance; this is a serious emotional [illegible]

if it becomes a general cultural characteristic, it must have a very serious social influence. I am very, very glad that you brought that out because again it brings in the interdiscipline nature of all our problems, and the fact that we have to have this breadth of viewpoint if we are going to see the full significance.

Knisely. To generalize, for instance, this instantly raises the question of nutritional states of various castes of India and what happens to children of such ages throughout China

Best· I am working on Dr Fremont-Smith to have the next meeting of the Liver Conference in Uganda

Fremont-Smith: That would have a social impact on us, which would be very good for us I am sure.

Davies I hope that stays in the record. I think too that this is made worse by another factor with which it is closely linked, namely, the fact that weaning takes place at about 18 months to two years. It must be a tremendous blow to the child's mentality when at a year, 18 months or two years he is suddenly removed from the mother's breast and replaced by another child, especially when it is followed by a period of prolonged invalidism (see Williams(60))

To go further into this will lead into very difficult fields of psychology about which I know very little All I would be able to say is it must have a tremendous impact upon African social life in general, and possibly explains to a great extent what we are accustomed to talking about, the tropical backwardness It may be a great impediment to development

Fremont-Smith May I ask one question What is the attitude of the parents, particularly the mothers and the adults of the family during the period immediately after weaning? What I am asking is are the children loved and do they get a lot of affection? Is there permissive attitude or what is the relationship, because that would be a very crucial aspect of the situation?

Best: I don't know what that is.

Davies: I prefer to call a Laennec cirrhosis, a pseudo-monolobular cirrhosis because although there is an appearance of individual lobules surrounded by fibrous tissue there is, in fact, no true architecturally normal lobules because the fibrous bands link central vein and portal tract, central vein and central vein, or course irregularly across areas of the lobule In a biliary cirrhosis the individual lobules may be outlined by fibrous tissue demarcating an architecturally normal lobule. It is a true monolobular cirrhosis.

Best. I think we ought to go on with the second part of your presentation.

Davies· I now want to talk about certain features of this disease which we think make it so important all the world over. I know this Conference has always taken a broad view of what constitutes a discussion relative to liver injury and I think what I have to say is closely linked to liver injury. I want to try to relate it to certain factors and conditions which are operative, particularly in Uganda, but, I think, as Dr. Gyorgy has pointed out, all over the tropical world, and which today assume very great importance. One thing I did not mention before, but which I think is of extreme importance is the mental state in kwashiorkor.

One of the first signs of this disease shown by the children is an extraordinary change in their mentality(7, 11). From being normal happy children they become peevish, irritable, whining photophobic children, often crying in misery and just wanting to be left alone, apathetic and anorexic. They present, in fact, a mental state very similar to that seen in severe cases of pink disease(11, 59) and this state persists to a greater or lesser extent between the ages of 18 months and four years, during the acute stage of kwashiorkor. Thus in this period when the more happy child in temperate regions is first making contact with the world about him, actively exploring it, making his first social contacts, learning perhaps faster than he ever learns in his later life, at that very period the majority of African children are peevish, whining invalids with no interest in the world around them This is the picture perhaps in the severe case, but in mild cases you see the same picture. I am no psychologist, but I think this must impose a tremendous psychological handicap on the whole of tropical Africa and perhaps in other regions as well. That is one reason why I think this disease is so important

of the frequency of unexplained parotid swelling and this may be a manifestation of the same state of affairs(62).

This leads me to a particularly horrifying conclusion(63), namely, that millions of Africans and other tropical dwellers are already so seriously damaged by this form of malnutrition that even if they obtained a diet, fully adequate in all respects, they could never be restored to full health It underlies the necessity for a full exploration of this disease for each year the problem gets worse as more are damaged May I say that no one would be more pleased than I if all my conclusions were shown to be erroneous.

When we come to the liver, we think that this also is permanently affected by a long period of fat infiltration. I have previously men- [illegible]

sider persisting liver damage, I want to discuss briefly some features of general African pathology.

Shorr: Have any studies been made on the feeding of pancreatic digests?

Davies. We thought of it. We have not done it We thought of using papain but have not done so

In considering the general pathology of Africans we have to remember that they die young These figures (Table II) are from the Mulago Hospital Autopsy Records of 3256 autopsies(64), a series subject to every possible defect of selection But they are the

TABLE II

AGE AT DEATH OF AFRICANS AUTOPSIED AT MULAGO HOSPITAL, 1931-1949

Age in Years	11-20	21-30	31-40	41-50	51-60	61-70	over 70	"old"
Number of autopsies	452	1249	795	529	168	34	4	25

only large series of figures bearing on this aspect of pathology known to me from East and Central Africa You will observe that the vast majority of these cases die young. I found the average age at death in this series to be about 28½ years, so you would not expect certain diseases we associate with old age to be common

Davies: That is a very important question, sir. I only wish I could answer it in greater detail but it is a field about which I know very little. My impression is that the African is extremely fond of children, extremely kind to children, but of course, the way of life is so very difficult in some respects that when another child comes along the mother is almost bound to devote her attention to the new child in order to give it a chance to survive. (Dr. Cecily Williams(60) paid great attention to this aspect of the disease.) I think the child must feel bitterly the withdrawal of attention and its concentration on its successor. The attitude towards the child when he is ill is, I think, as sympathetic as it could be, but the trouble is that the disease is looked upon as a part of the normal process of growing up, almost as was rickets in England two hundred years ago.

Leaving that aside, the next thing I should like to mention is that we have some very suggestive evidence that if the pancreatic and intestinal and liver damage is not speedily relieved persistent malfunction results. Some of this evidence comes from some work done by Dr. Kekwick(61) in the East African Army during the last war, unfortunately not published in full. These African troops were picked out from among the healthiest members of their tribes after a physical examination But physical efficiency tests showed that as compared to European troops their efficiency was considerably less. Despite a very excellent diet which was consumed in very considerable amounts it proved impossible to build them up to European levels of physical efficiency. In part this was explicable on the grounds that they simply were not digesting and absorbing a large part of this excellent diet In fact, there was a relatively low limit to their digestive capacities explaining a fact similar to all workers in Africa, the very bulky stools of Africans and their high content of undigested food, reflecting in large measure what goes in the mouth. A sprue syndrome is not seen but feeding fat produces steatorrhea, feeding meat, creatorrhea and all the time there is undigested carbohydrate Of course all these vary in amount from case to case. So we suppose that unless the pancreatic and enzyme secreting cell defects are speedily relieved the victims are in some way, as yet unexplained, prevented from ever attaining full functional efficiency; this possibly explains their liability to further attacks of kwashiorkor This may well be true for the parotid glands; we know they atrophy in the acute stages and we have seen swelling in response to treatment. There are many reports from the tropics

of the frequency of unexplained parotid swelling and this may be a manifestation of the same state of affairs(62).

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When we come to the liver, we think that this also is permanently affected by a long period of fat infiltration. I have previously mentioned what I believe to be histologic evidence of this. Now I want to discuss this proposition in relation to African pathology generally [illegible]

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Best: I don't understand why you didn't have more deaths in the infant group?

Davies: I should say that I have deliberately excluded all children dying under the age of ten years. Despite this the average age at death was just over 28 years. These young children do not die of parasitic disease as a rule(65). *If there is one thing that has impressed me while doing autopsy work in Uganda it is of the relative unimportance of tropical parasites in bringing cases to autopsy, however much they may open the body to serious defect or cause invalidism* The commonest causes of childhood death in Uganda are malnutrition and acute and chronic infections. In fact, the majority of children die, apart from malnutrition, from the same diseases a clinician sees in any temperate-region hospital.

I have analyzed a group of 229 adults with congestive cardiac failure(65) and can now answer Dr. Shorr's question. *We do not* [illegible] quite uncommon. [illegible] grade, secondary [illegible] nd acute bacterial endocarditis *are common. Rheumatic heart disease* – that means mitral stenosis as far as we are concerned because we rarely see acute rheumatism(68) – is sometimes encountered. I wonder if it is always rheumatic in origin in Africans. Eight of these patients dying with congestive failure had no cardiac lesions but marked cirrhosis of the liver That is a peculiarity which seems to crop up repeatedly, the difficulty of distinguishing between congestive cardiac failure *in Africans and cirrhosis. We think there may be a* connection. Let me put it this way, I think there may be a congestive cardiac failure due to cirrhosis.

Watson: I did not understand you. You think the cirrhosis causes the congestive heart failure?

Davies. [illegible] ently go together, relatively [illegible] distinguish. I am not talkin[illegible] stive failure associated with a Laennec-type cirrhosis and perhaps more frequently with postnecrotic scarring(69)

Watson. You are talking in terms of previous *myocardial injury* which you mentioned?

Davies. No, although that association also crops up In this series of cases of congestive failure there were 21 cases of endomyocardial necrosis, and 13 cases with no cause found

Knisely: Why is that so small?

Davies: Which group?

Knisely. No cause found Sad to state, "no cause found" is the most popular form of death in many places

Davies. I grant you that

Knisely: Even though we look.

Davies: We have looked, but after all the common causes of failure have been eliminated, the pathologist is beginning to get stuck. We do see a number of cases of myocarditis of a type resembling Fieldler's myocarditis

Shorr· Could I ask whether, apart from the renal disease that you say is so common, there is clinical evidence of oliguria in this population?

Davies: Antidiuresis?

Shorr· How rapidly do they excrete a water load?

Davies: We have not looked into it

Fremont-Smith. How much edema and ascites do they have?

Davies. Both, in tremendous amounts They often go together That is one of the things that makes the differentiation between cirrhosis and myocardial failure so difficult When we think it is the one it is often the other

Hartroft. What is the age-incidence of patients with renal hypertension?

Davies. I think the majority fit into the 20-30 age group

Watson You mean mostly chronic glomerular nephritis?

Davies No, urethral stricture, etc giving rise to secondary hypertension

Best What do you mean by hypertensive renal disease?

Davies I mean a disease in which the first damage is to the kidney, not following a prolonged period of raised blood pressure

Watson Not primary?

Davies Not primary hypertension

Shorr Pyelonephritis?

Davies Pyelonephritis urethral stricture with or without infection or hydronephrosis this curious paraamyloidosis of the kidneys.

a whole variety of renal diseases(70). Atheroma as a cause of death we rarely see. In twenty years of autopsy work at Mulago by my predecessors, my colleagues and I have only seen it occasionally(65).

While we do not see it commonly, apparently they do see it quite commonly in South Africa I would be interested to know where between Kampala and Johannesburg the rise takes place(71). Perhaps connected with this is the rarity of postoperative thrombosis and pulmonary embolism at Mulago Hospital (African) as compared with the European Hospital in Kampala. I might mention here the high incidence of pneumococcal infections. Pneumococcal meningitis is the commonest type of acute meningitis and was so before the days of chemotherapy(72). In any form of septicemic condition, where in Europe one would think of the streptococcus hemolyticus, in Uganda one must think first of the pneumococcus; this too may be related to malnutrition and liver damage. Pneumococcal pneumonia and other pulmonary infections are common and cause a great many deaths(72, 73) while the appalling severity of pulmonary tuberculosis makes it a dreadful problem(74). On an average they die in about six months from the first symptoms of the infection, with an extremely acute primary form of lesion

But against this background of disease I want to return again to the question of persistent liver damage. The sensitivity of the African to hepatotoxic drugs is notorious(35) but I want to raise the problem of a more subtle form of derangement, namely, the problem of sex hormone imbalance leading to hyperestrogenism The damaged liver cannot, it is claimed, inactive estrogens(75) and it is suggested that these mount up in the body and give rise to hyperestrogenic effects(76). The presence of an actual cirrhosis is not necessary(77) and these changes may be seen in protein deficiency(78) or with lipotropic factor deficiency states(79). Since every African appears to have a damaged liver one might expect to see the consequences of hyperestrogenism to be widespread in Africa, as indeed I think is true In a recent survey of African railway workers in Nairobi, Dr. Trowell found that between three and five percent of these supposedly healthy workers had gynecomastia(9a) a figure fantastically at variance with those reported from temperate regions

Elsewhere(80) I have noted the high incidence of carcinoma of the breast in African males, for in Uganda of 64 carcinomas 5 were in males. In Europe and Australia the incidence in males is about

2% of breast carcinoma, in Africa various reports quote figures between 7 8% to over 25%

Fremont-Smith: Invasive type?

Davies. Yes.

Fremont-Smith: Not benign?

Davies: Really malignant carcinoma and besides this, there is apparently a high incidence of sarcoma of the breast as well So even these figures underestimate the total malignancy in male breasts Again, it is impossible to sex the bones of Africans by secondary sex characteristics The anthropologists have long referred to the Bantu as a people showing "castration effects"(81). Their skins in health are beautifully soft and satiny, the hair has often a feminine distribution and the males often have a feminine body outline(82). I could recite a long catalogue of things which seem to me to indicate that "estrogenization" due to liver damage is a most potent factor influencing pathologic conditions in Africa(70)

Hanger. Do they have spiders, nevi in the skin?

Davies: No Nor do they get interstitial cell tumors of the testes

Shorr: Do the women have menstrual irregularities and functional menorrhagia? What about their fertility?

Davies: I cannot tell about the fertility The only endometrial biopsies I see are selective I do not know I do think the incidence of body to cervical cancer of the uterus is disproportionate to the incidence in temperate regions, especially considering the greater ease of diagnosis of cervical cancer under primitive conditions The figures in Uganda for cancer of the uterus were body 22, cervix 37, roughly a proportion of 2 3, whereas in temperate regions the proportion is regarded as 1 4 or even 1 9

It is also said that in primitive communities sarcoma is much more frequent than carcinoma is in temperate regions This is not true in Uganda If all the reticulo-endothelial tumors, lymphosarcoma, reticulum cell sarcoma, etc , are taken away from the genuine sarcomas, the incidence of sarcoma to carcinoma is not greatly disproportionate to the figures in Europe But the incidence of sarcoma in the tropics is high because of the great numbers of reticulo-endothelial sarcomas(83) The fact that these are known in animals to be powerfully influenced by estrogens may be of significance(84) I do not claim that this is more than speculation but it does seem to me to be particularly interesting that so many

and interest has been aroused in the pseudohypophysectomy syndrome of Mulinos and Pomerantz(87). The pituitary of Africans has recently been described by Vint(88) in a preliminary communication. He has reported the results of Rasmussen counts on African pituitaries and has found that in normal Africans there is *an increased acid* [illegible] which he considers of [illegible] would draw your atten[illegible]nt (in *male Africans) to those found by Rasmussen in the white female."* This seems to me to be a statement of great importance.

Now I would draw your attention to another point, namely, the frequency of carcinoma and other malignant tumors in Africans occurring in the pancreas, small intestines and salivary glands(89). These, too, show a remarkable incidence in Africans as compared to Europeans and it is perhaps worthy of passing note that these organs, so heavily damaged by kwashiorkor in infancy, should so frequently be the site of cancer in later life. Then, and this is, I admit, a most unscientific way of going about things, but in 1300 cancers there were three carcinoma of the lachrymal gland, a quite disproportionate figure. It makes me suspicious that the lachrymal gland is involved in kwashiorkor.

Watson: Is the pancreas very small?

Davies: Relatively small

Watson. I think the incidence as compared with the liver carcinoma is out of proportion with what we see.

Davies: I agree.

Watson. The stomach incidence seems very low.

Davies Yes, the stomach figure is very low, but lymphosarcoma of the stomach and small intestine is not uncommon But I don't want to stress this I admit the figures just do not compare with those available from the temperate regions. But they are the only ones available from this area. I think that the surgeons, particularly in recent years, have been sending us a genuine cross-section of everything they see and that this is what they see

Patek Dr. Davies, you stated that despite the vast amount of cirrhosis in Africa you saw little evidence of portal hypertension — no deaths from varices This is very unusual. Is there anything distinctive about the spleens in your cases?

Davies: When it comes to an African spleen, well, one talks about the African liver being a histological nightmare, you should see the spleen! It has been malarious from infancy and very often it has been host to many other parasites. It is so fibrosed and altered that I find it very difficult to understand. We do see a lot of splenomegaly and lately it has been bulking rather importantly in our minds. Largely due to the rise of modern chemotherapy, Africa is now seen to be full of mysterious diseases missed in the past because they were assumed to be the results of parasitic infestation Thus, in recent years, a considerable number of new diseases have been recorded from Africa, onyalai, tropical phlebitis and primary gas-filled splenic abscess, all recently reviewed by Gelfand(90, 91) rift, valley fever, endomyocardial necrosis, symmetrical gangrene of the extremities and others, and no doubt there are many more lurking behind the mask of parasitism We are becoming increasingly aware of a disease first described in Nyasaland by Leys(92) in which young adults come up with enormous splenomegaly of five to six years' duration and they die with cirrhosis of the liver and ascites and hepatic failure

Hanger: They do that in New York City

Gyorgy: And in Philadelphia

Davies. They do it a lot in Egypt It has been attributed to schistosomiasis and I daresay this is true in the majority of cases but there is an appreciable residuum in which I think the cause is not schistosomal(93) We have some evidence that when the spleen is seriously affected the liver is normal and if you take the spleen out they do well

Gyorgy. The same in New York and Philadelphia

Watson Say that again, please

Davies: If you take the spleens out they apparently do quite well If you leave them in they die

Watson· The Banti syndrome

Gyorgy: Pediatricians called it in the past splenic vein thrombosis

Davies: No, we have looked for that

Gyorgy· I agree, the term "splenic vein thrombosis" is a misnomer; thrombosis is rarely found It is probably better to call it simply Banti syndrome

remains in, they die later of cirrhosis. But I cannot see how it all can possibly be explained on the schistosomiasis. It is clear a great many don't have schistosomiasis.

Schiff: What do you think actually happens?

Davies: I don't know. We only recently have come upon this. Ten years ago it was all called malaria.

Watson: May I mention briefly a case in point that I saw just a few years ago in a young man, about 25, with massive splenomegaly who had some laboratory evidences of liver functional impairment, bromsulfalein retention, no elevation of serum bilirubin, considerable urobilinogenuria. Shortly after this study he had massive hematemesis and died. At autopsy the spleen showed diffuse fibrosis without known cause We were unable to find anything in the splenic vein or in the portal vein, and the liver was not cirrhotic, and histologically the liver looked normal We could not prove that there was any evidence of hepatic disease and yet he had clear cut clinical evidence of hepatic functional impairment with the picture of Banti's syndrome, if you will.

Schiff: That case I showed yesterday had laboratory evidence of liver involvement. Biopsy, however, showed no liver involvement. How often have you seen a case of congestive splenomegaly, where you were sure there was no liver involvement at the time of splenomegaly, develop, in the course of time, cirrhosis of the liver?

Watson. I don't believe I have a proven case of that type.

György: It is chiefly a pediatric problem I have seen at least three children in whom splenomegaly with hematemesis or melena was observed between three and eight years of age. Years after splenectomy, cirrhosis, and jaundice followed, with a fatal outcome. There is often widespread pigmentation of the skin.

Schiff: I was unaware of that although I know Banti so contended in his original communication

Hanger: The more I see of idiopathic splenomegaly, the more I feel it should not be confused with a similar picture produced by portal hypertension.

Davies: It is being confused because too much stress is laid on purely morphological interpretations. I think that is part of the mistake. It is perhaps worth bearing in mind that much of the

classic work on splenic and hepatic disease by Hanot, Banti and others was done at a time when no doubt the peoples among whom they worked were much more poorly nourished than now. The diseases seen in the French and Italian peasantry of those days may have been very similar to the diseases we are now seeing in malnourished peasants in Africa. I think we are seeing conditions remarkably similar to those described by Hanot, Banti and others.

Gordon. I would like to comment briefly on the mathematical balance sheet of these lactating women that Dr. Davies mentioned It really is a metabolic curiosity It seems to me during the later part of that lactating period these women must put out 35-40 grams of protein in 24 hours and they need an additional 15-20 for their own economy.

Gyorgy: How much did you say?

Gordon: 35-40 grams in the milk in 24 hours for a child of two. They don't get anything in addition to breast milk It means these women must need 60-65 grams of protein

Gyorgy: According to the recent report of Macie-Hoobler and her group, the protein content of breast milk is about 1-1.25%; thus one quart of breast milk contains about 10-12 5 gms of protein

Gordon. I stand corrected With the absorption defects that these people have, the extremely limited diet, the poor quality of protein that they have, it seems almost incredible that they could go on lactating with as great facility as you say they do Even if they did I should think it would be at the expense of all their own body tissues. It is an amazing thing

Davies. I think that is quite true It is amazing that they can go on lactating, but it is equally amazing what a tremendous empty reservoir of protein they have I know my colleagues in Uganda, particularly Dr. Eric Holmes, have been investigating the protein reserves in these patients I do not know Dr Holmes' final results and must not anticipate his report, but I am sure he will allow me to say that they appear to have an enormous protein deficit which takes a very long time to balance However, lactation in African women is an amazing process, for apparently you can make any African woman lactate, aunt, grandmother sister or any woman and they can be made to lactate in a few days Reliance is placed on warmth, massage, the sucking of the child and various herbs (60, 98, 99).

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probelms, and if any of you are ever near Uganda we should like you to pay us a visit and see the problems at first hand.

Thank you very much.

Best: It is a really beautiful place. We thank you, Dr. Davies. You have added greatly to our program.

REFERENCES

1. WATERLOW, J *Fatty Liver Disease in Infants in the British West Indies.* London Medical Research Council Special Report No. 263, 1948
2. a) VÉGHELYI, P V. In discussion on nutrition, Internat. Congress of Pediatrics 1947 *Acta. Pediat.* 36, *128 (1948)*
   b) ———: Pancreatic function in nutritional oedema. *Lancet* 1, *497 (1948).*
3. STUBBS, E. S V Oedema in East Africa. Oedema in food-deficiency diseases In Bigland A D *Lancet* 1, *245 (1920)*
4. PROCTER, R A W Medical work in a native reserve. *Kenya, Med J* 3, *284 (1927)*
5. KÉRANDEL, J · Sur la bouffissure d'Annam et son pathogenie *Bull Soc. path exot* 19, *302 (1926).*
6. NORMET, L · La "bouffissure d'Annam" *Bull Soc. path. exot.* 19, *207 (1926)*
7. WILLIAMS, C. D · Nutritional disease of childhood associated with maize diet. *Arch Dis Childhood* 8, *423 (1933).*
8. ——— *Kwashiorkor; nutritional disease of children associated with maize diet Lancet* 2, *1151 (1935).*
9. a) TROWELL, H C Medical examination of 500 African railway workers *East African M J* 25, *236 (1948).*
   b) ———. Malignant malnutrition (kwashiorkor) *Tr Roy. Soc Trop Med & Hyg* 42, *417 (1948)*
10. SMITH, E C (Lagos, Nigeria) Child mortality in Lagos, Nigeria. *Tr Roy Soc Trop Med. & Hyg* 36, *287 (1943).*
11. TROWELL, H C Pellagra in African children *Arch Dis. Childhood* 12, *193 (1937)*
12. STANNUS, H. S Nutrit[illegible] of childh[illegible] with maize diet and pellagra.[illegible] *Childhood* [illegible] 34).
13. ———· Pell[illegible] conditi[illegible] nates. Tr[illegible] *All* 33, [illegible]
14. [illegible] D: Wh[illegible] in chil[illegible] Soc. [illegible] 34, *85* [illegible]
15. · Note [illegible] 13 [illegible]

16 HUGHES, W.: Achromotrichia in tropical malnutrition. *Brit. M. J.* 2, 85 *(1946).*

17 GILLMAN, J, and GILLMAN, T.: Liver disease in Johannesburg, relation to pellagra *Lancet* 1, *169 (1948)*

18 DAVIES, J N P. Essential pathology of kwashiorkor. *Lancet* 1, *317 (1948).*

19 a) TROWELL, H. C.: Infantile pellagra *Tr. Roy Soc. Trop Med. & Hyg.* 33, 389 *(1940)*

b) ——— Certain observations on kwashiorkor and pale-skinned oedemas seen in the Congo *East African M J.* 20, *132 (1943)*

c) LATHAM, D. V. Gillan's oedema *East African M. J.* 11, *358 (1935).*

d) GILLAN, R U Investigation into certain cases of oedema occurring among Kikuyu children and adults *East African M J.* 11, 88 *(1934).*

20 TROWELL, H C, and MUWAZI, E M. K Severe and prolonged underfeeding in African children, kwashiorkor syndrome of malignant nutrition *Arch Dis. Childhood* 20, 110 *(1945).*

21. ———, and ——— Contribution to study of malnutrition in Central Africa, syndrome of malignant nutrition *Tr Roy. Soc Trop Med & Hyg* 39, 229 *(1945)*

22 HARTZ, P H Pancreatic atrophy in infants with fatty liver. *Doc. Neer et Indon de Morb Trop* 1, *41 (1949)*

23 GILLMAN, J Effects on rats of prolonged feeding with the staple African diet *Brit M J* 1, *149 (1944)*

24 GILBERT, C, and GILLMAN, J Diet and disease in Bantu *Science* 99, 398 *(1944)*

25 KRISTAL, J Cystic and other changes in pancreas of rats fed diet of mealie meal and sour milk *South African J M Sc* 12, *47 (1947)*

26 VEGHELYI, P V, *et al* Dietary lesions of pancreas *Am J Dis Child* 79, *658 (1950)*

27 GILLMAN, J, GILBERT, C, and GILLMAN, T Bantu salivary glands in chronic malnutrition with brief consideration of parenchyma interstitial tissue relationship *South African J M Sc* 12, 99 *(1947)*

28 ELIAS, H A re-examination of the structure of the mammalian liver The hepatic lobule and its relation to the vascular and biliary systems *Am J Anat* 85, 379 *(1949)*

29 FIESSINGER, N *Les Ferments des Leucocytes, en physiologie pathologie et thérapeutique générales* Paris Masson, 1923

30 BERGEL, S Beziehungen der Lymphocyten zur Fettspaltung und Bakteriolse *Münch med Wchnschr* 57, *1638 (1910)*

31 BARNES, J M Enzymes of lymphocytes and polymorphonuclear leucocytes *Brit J Exper Path* 21, *264 (1940)*

probelms, and if any of you are ever near Uganda we should like you to pay us a visit and see the problems at first hand.

Thank you very much.

Best: It is a really beautiful place. We thank you, Dr. Davies. You have added greatly to our program.

REFERENCES

1. WATERLOW, J · *Fatty Liver Disease in Infants in the British West Indies*. London: Medical Research Council Special Report No 263, 1948.
2. a) VÉGHELYI, P V: In discussion on nutrition, Internat. Congress of Pediatrics 1947. *Acta Pediat.* 36, *128 (1948)*.
   b) ———· Pancreatic function in nutritional oedema *Lancet* 1, *497 (1948)*.
3. STUBBS, E S.· V. Oedema in East Africa. Oedema in food-deficiency diseases In Bigland A. D. *Lancet* 1, *245 (1920)*.
4. PROCTER, R A. W. Medical work in a native reserve. *Kenya, Med J* 3, *284 (1927)*.
5. KÉRANDEL, J.. Sur la bouffissure d'Annam et son pathogenie. *Bull Soc. path exot.* 19, *302 (1926)*
6. NORMET, L.: La "bouffissure d'Annam" *Bull. Soc. path. exot.* 19, *207 (1926)*
7. WILLIAMS, C D.. Nutritional disease of childhood associated with maize diet *Arch Dis Childhood* 8, *423 (1933)*.
8. ———. Kwashiorkor; nutritional disease of children associated with maize diet *Lancet* 2, *1151 (1935)*.
9. a) TROWELL, H C Medical examination of 500 African railway workers *East African M. J* 25, *236 (1948)*.
   b) ——— Malignant malnutrition (kwashiorkor). *Tr. Roy Soc Trop Med & Hyg*. 42, 417 *(1948)*
10. SMITH, E C. (Lagos, Nigeria) Child mortality in Lagos, Nigeria. *Tr. Roy Soc Trop. Med & Hyg* 36, *287 (1943)*.
11. TROWELL, H C Pellagra in African children. *Arch Dis. Childhood* 12, *193 (1937)*
12. STANNUS, H S Nutritional disease of childhood associated with maize diet and pellagra *Arch Dis Childhood* 9, *115 (1934)*.
13. ——— Pellagra and pellagra-like conditions in warm climates. *Trop Dis Bull* 33, *729 (1936)*
14. WILLIAMS, C. D What is pellagra in children? *Tr. Roy. Soc. Med & Hyg* 34, *85 (1940)*.
15. TROWELL, H C Note on infantile pellagra *Tr. Roy Soc. Trop Med & Hyg* 35, *13 (1941)*

16 HUGHES, W.. Achromotrichia in tropical malnutrition. *Brit. M. J.* 2, 85 *(1946).*

17. GILLMAN, J, and GILLMAN, T: Liver disease in Johannesburg, relation to pellagra *Lancet* 1, *169 (1948).*

18 DAVIES, J N P.: Essential pathology of kwashiorkor *Lancet* 1, *317 (1948).*

19 a) TROWELL, H C.: Infantile pellagra. *Tr. Roy Soc Trop. Med. & Hyg.* 33, 389 *(1940)*

b) ——— Certain observations on kwashiorkor and pale-skinned oedemas seen in the Congo *East African M J.* 20, *132 (1943)*

c) LATHAM, D V.: Gillan's oedema *East African M. J.* 11, *358 (1935).*

d) GILLAN, R. U: Investigation into certain cases of oedema occurring among Kikuyu children and adults *East African M J* 11, *88 (1934).*

20 TROWELL, H C, and MUWAZI, E M K. Severe and prolonged underfeeding in African children, kwashiorkor syndrome of malignant nutrition *Arch Dis Childhood* 20, *110 (1945)*

21 ———, and ———. Contribution to study of malnutrition in Central Africa, syndrome of malignant nutrition *Tr. Roy Soc Trop Med. & Hyg* 39, *229 (1945).*

22 HARTZ, P H Pancreatic atrophy in infants with fatty liver *Doc Neer et Indon de Morb Trop* 1, *41 (1949)*

23 GILLMAN, J Effects on rats of prolonged feeding with the staple African diet *Brit M J.* 1, *149 (1944)*

24 GILBERT, C, and GILLMAN, J Diet and disease in Bantu *Science* 99, *398 (1944).*

25 KRISTAL, J Cystic and other changes in pancreas of rats fed diet of mealie meal and sour milk *South African J M Sc* 12, *47 (1947)*

26 VÉGHELYI, P V, *et al* Dietary lesions of pancreas *Am J Dis Child* 79, *658 (1950)*

27 GILLMAN, J, GILBERT, C, and GILLMAN, T Bantu salivary glands in chronic malnutrition with brief consideration of parenchyma-interstitial tissue relationship *South African J M Sc* 12, *99 (1947)*

28 ELIAS, H A re-examination of the structure of the mammalian liver The hepatic lobule and its relation to the vascular and biliary systems *Am J Anat* 85, 379 *(1949)*

29 FEISSINGER, N *Les Ferments des Leucocytes en physiologie pathologie et térapeutique générales* Paris Masson, 1923

30 BERGEL, S Beziehungen der Lymphocyten zur Fettspaltung und Bakteriolse *Munch med Wchnschr* 57, *1638 (1910)*

31 BARNES, J M Enzymes of lymphocytes and polymorphonuclear leucocytes *Brit J Exper Path* 21, *264 (1940)*

32 VINT, F. W.: Cirrhosis of liver in East African native *Kenya & East African M J. 7, 349 (1931)*

33 BEST, C. H.: *Diabetes and Insulin and the Lipotropic Factors.* Springfield, Ill: C. C Thomas, 1948.

34 HIMSWORTH, H P. *Lectures on the Liver and its Diseases.* Harvard Harvard Univ. Press and Oxford: Blackwell, 1947.

35. CULLINAN, E R.: Medical disorders in East Africa. *Tr Roy. Soc. Trop. Med & Hyg 39, 353 (1946).*

36 GILLMAN, J., *et al* · Production of severe hepatic injury in rats by prolonged feeding of maize-meal porridge (mealie-pap) and sour milk *Brit J Exper. Path 26, 67 (1945).*

37 ROBERT, L, *et al* · Une épidémie de hériberi à forme humide observée à Dakar en 1932 *Bull Soc path. exot 26, 512 (1933).*

38 HENNESSEY, R S F Observations on nephritis in Uganda natives *East African M J 15, 329 (1938)*

39 NORMET, M L Sur la pathogenie de la bouffissure d'Annam, maladie de carence *Bull Acad Med Paris* 117, *239 (1937).*

40. BEDFORD, D E, and KONSTAM, G L. S Heart failure of unknown aetiology in Africans *Brit Heart J 8, 236 (1946)*

41 DAVIES, J N P Endocardial fibrosis in Africans *East African M. J. 25, 10 (1948)*

42 ——— *Endomyocardial Necrosis* M D Thesis Bristol. 1948. (To be published)

43 HAWES, R B Treatment of acute fulminating cardiac beriberi (shossin) *Tr Roy Soc Trop Med & Hyg* 31, *474 (1938)*

44 SMITH, J J, and FURTH, J Fibrosis of endocardium and myocardium with mural thrombosis, notes on its relation to isolated (Fiedler's) myocarditis and to beriberi heart *Arch Int Med* 71, *602 (1943)*

45 DICK, G W A Aortic size in East African natives *Ann Trop Med* 41, *52 (1947)*

46 RAMALINGASWAMI, V, MENON, P S, and VENKATACHALAM, P S Infantile pellagra, report on 5 cases *Indian Physician 7, 229 (1948).*

47 HUGHES, W: Kwashiorkor and ariboflavinosis *Tr Roy. Soc Trop Med & Hyg 39, 437 (1946)*

48. ALTMANN, A Syndrome of malignant malnutrition (kwashiorkor infantile pellagra), its conception as protein deficiency and its treatment with skimmed lactic acid [illegible] Proc. 7, 32

51 CULWICK, G. M.: Nutrition in East Africa. *Africa* 14, 401 (1943).

52 PLATT, B S: Tables of representative values of foods commonly used in tropical countries. *Med. Res. Council Spec. Report* No 253, 1945

53 ALTMANN, A, and MURRAY, J F.: Anaemia of malignant malnutrition (infantile pellagra, kwashiorkor); protein deficiency as possible aetiological factor. *S. African J. M Sc.* 13, 91 (1948)

54. LEHMANN, H. L: Macrocytic anaemia in Central Africans in relation to ancylostomiasis and other diseases. *Lancet* 1, 90 (1949).

55. JACKSON, C M.: *The Effects of Inanition and Malnutrition upon Growth and Structure* Philadelphia Blakiston, 1925.

56 BLOCH, C. E.: Die Säuglings-Atrophie und die Paneth'schen Zellen. *Jahrb. f. Kinderh* 59, 1 (1904)

57. ARRAGA, A, and VINAS, M Sclérose du pancréas consécutive aux gastro-enterites chroniques. *Arch. de Méd. des Enf* 3, 402 (1900)

58 KINNEY, T. D, HEGSTED, D. M, and FINCH, C A: The influence of diet on iron absorption I The pathology of iron excess *J Exper Med* 90, 137 (1949)

59 ROCAZ, C *Pink Disease (Infantile Acrodynia)* Trans by I J Wood London Hopkinson, 1938

60. WILLIAMS, C D: Child health in Gold Coast *Lancet* 1, 97 (1938).

61. KERWICK, A Dietary survey of Kings African Rifles Unpublished data quoted by Trowell, H. C: Medical examination of African railway workers *East African M J* 25, 426 (1948)

62 WENCKBACH, K. F St Cyres lecture on heart and circulation in tropical avitaminosis *Lancet* 2, 265 (1928)

63. DAVIES, J N P Causes of sudden death in Africans *East African M J* 25, 322 (1948)

64 ——— Pathology of central African natives. Mulago Hospital post mortem studies *ibid*. 24, 180 *May* 223 *June*; 289, *Aug* (1947)

65. ——— Cardiovascular diseases in Africans *ibid* 25, 454 (1948)

66 WILLIAMS, A W Blood pressure of Africans *East African M J* 18, 109 (1941)

67 ——— Heart disease in native population of Uganda, hypertensive heart disease *ibid* 21, 328 (1944)

68 ——— Heart disease in native population of Uganda, rheumatic heart disease *ibid*. 16, 341 (1939)

69 LOEPER, M *Les Hépatites* Paris Masson, 1937.

70 DAVIES, J N P Renal disease in Africans *East African M J*. 26, 76 (1949)

SECTION VI

VITAMIN B_{12}, FOLIC ACID, AND THE LIPOTROPIC AGENTS

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I DON'T claim that I will bring all pertinent data and discuss them. First of all, the hour is late. Second, I have no firsthand knowledge of various new developments, and finally, I am flanked right and left by people who could comment in many respects better, especially on the enzymatic problems involved, than I.

The rather complicated interrelations between the various dietary factors involved in the production or prevention of massive necrosis or cirrhosis of the liver are summarized in Table I.

TABLE I

	EFFECT ON	
Dietary factors	Necrosis	Cirrhosis
Protein (methionine containing)	Beneficial	Beneficial
Methionine	Beneficial	Beneficial
Cystine	Beneficial	Injurious
Choline	0 or injurious	Beneficial
Vitamin E	Beneficial	0
Fat	0 or injurious	0 or injurious
B_{12}	0	Beneficial (?)
Antibiotics	Beneficial	?

In cirrhosis choline, and as Dr. Best calls it, its precurser, methionine or methionine-containing proteins, are the best-known beneficial dietary factors As you will remember, several years ago the late Dr Hoagland and his associates(1) recommended for the treatment of cirrhosis in man crude liver extract given intravenously. No explanation was ever given for the necessity of injecting crude liver extract intravenously and for a while even no explanation was

given why liver extract should be effective. Hoagland's collaborators, Labby and Ralli and his and her colleagues claimed that perhaps liver extract is effective because it increases the appetite of cirrhotic patients.

I became interested in this problem and tried liver extract in the prevention and especially also in the treatment of experimental dietary cirrhosis. In a publication about a year ago(2) we concluded that liver extract given by
peutically effective, especially in
of choline or methionine (protein
liver extract might have contained choline, could not be excluded, although the fact that with an excess of methionine or protein liver extract was still found effective militated against this simple assumption

About the same time and independently Drill and his collaborators appeared to become also interested in liver extract as a lipotropic agent, and concluded that liver extract has a lipotropic potency unexplained by and beyond its content of choline as determined by the usual chemical analytical methods(3). More recently Drill and McCormick published observation on the use of a vitamin B_{12} concentrate, practically free from choline as lipotropic agent(4) They found it lipotropically active and they called attention to the fact that liver extract is the best and original source of B_{12}

In our own studies we used crystalline vitamin B_{12} as supplement (0.5γ daily) to a low protein-high fat diet as well as to a low protein-low fat ration We were able to show that B_{12} exerts a definite lipotropic effect also when given by mouth(5). The experiments further indicated that B_{12} in the amount given (0.5γ daily) has shown no lipotropic activity when given as supplement to a low protein-high fat diet, in contra-distinction to its distinct lipotropic activity in rats fed a low protein-low fat diet Under these original experimental conditions methionine was found to be more effective than vitamin B_{12} in preventing fat deposition in the liver In more recent studies we attempted to answer the question, whether with increased dosage of B_{12} the lipotropic effect may become evident in rats fed a low protein and high fat diet or whether the sparing effect of vitamin B_{12} could be made more evident with reduced suboptimal amounts of the lipotropic compound (choline, methionine).

Handler: This problem was always inherent in the concept of a "pool of labile methyl groups." Th[illegible] [illegible] shuttle back and forth be- [illegible] and homocysteine, e.g., take up a "methyl" group from some other dietary source such as the methylene groups of glycine and transfer it via methionine [illegible] [illegible] protein levels, the methyl groups of dietary choline were not effective for methylnicotinamide synthesis, unlike the methyl group of added dietary methionine. This is the essence of the nutritional aspect of the problem. As Dr. Gyorgy quite properly noted, while Stekol's experiments certainly proved the availability of the carbon of glycine and serine for "methyl" synthesis, this was only demonstrable in the presence of dietary homocystine, which never occurs in natural foodstuffs But Dr. Gyorgy's data has indicated the lipotropic effect of B_{12} in a diet to which no homocystine had been added.

Gyorgy. Vitamin B_{12} has to fulfill quite a variety of functions in the animal and human body. We know from microbiological tests that B_{12} may be intimately connected with purine metabolism We know bacteria that require either B_{12} or desoxyribonucleosides. Thus the conclusion is probably warranted that B_{12} catalyses nucleoside-synthesis. In the absence of B_{12} the bacteria will then require exogenous supply of such nucleosides. We also know, — and that is for our special consideration much more interesting, — that animals kept on diets containing exclusively vegetable protein like soya bean and corn meal will grow very well, just as well as on any other diet containing animal protein, provided the diet containing only vegetable protein is supplemented with vitamin B_{12}. These findings may have a bearing on the world-wide problem of kwashiorkor and related conditions of malnutrition The recurrent etiologic nutritional factor in these conditions is, as seen also in the material collected in Jamaica, low protein intake, the protein being almost exclusively of vegetable origin, with no or only exceedingly small amounts of animal protein.

In order to increase the intake of protein and of vitamins in Jamaica, supplementation of the diet with food yeast, grown locally on molasses, was recommended during the last war by a special nutritional committee of the British Government. At that time it was not known that yeast does not contain vitamin B_{12}. Supple-

ments of yeast may beneficially influence all other possible vitamin B deficiencies, but might even accelerate or enhance, through disturbed equilibrium, vitamin B_{12} deficiency. According to Bean(10) dietary neuropathies, perhaps of the same type as seen so often in Jamaica, may benefit often dramatically, by vitamin B_{12}. In Jamaica, and as we heard today also in Uganda, and most probably everywhere where dietary liver injury (cirrhosis) is widespread, especially in infants and children, the diet must be low in vitamin B_{12}.

The practical conclusion is obvious. We should supplement the diet in Jamaica and in other parts of the world where similar dietary conditions prevail, with vitamin B_{12}. It is of paramount importance that this could be achieved without major changes in the dietary habits of the population and without food import. There would be no need to substitute animal proteins, usually unavailable in the relevant countries, for vegetable protein which is locally produced. Further B_{12} could also be produced by fermentation procedure, various strains of actinomyces being the best source of B_{12}. Culture media would also be available in tropical countries, for instance soy bean broth. Thus, the approach appears to be feasible, perhaps with the assistance of the World Health Organization or Point Four of Truman.

At our last Conference I referred to investigations in progress on the effect of aureomycin in the production of dietary massive necrosis(11). We found that aureomycin delayed the development of dietary necrosis. I mention this observation in conjunction with vitamin B_{12} for the following reason. The experimental diet on which necrosis regularly occurs (90-100%) in rats, consists of yeast as sole source of protein, starch, salt and vitamins. The vitamin supplements have not contained vitamin B_{12} and yeast, as already mentioned, is free from B_{12}. On the other hand we had to bear in mind that aureomycin is produced by streptomyces aureofaciens which also produces in large quantity vitamin B_{12}. Thus, aureomycin might easily be "contaminated" with B_{12} and the beneficial effect of aureomycin in massive dietary necrosis could have been due to this "contaminant" and not to aureomycin as such.

In our original experiments we used older rats, with an average weight of about 140 grams. At the end of 200 days 50% of the aureomycin rats were alive, whereas 90% of the control rats died from massive necrosis, starting from the 100th day of the experiment in increasing number, up to 170 days (Figure 1).

of dietary hepatic necrosis especially in comparison to that of aureomycin.

We tested penicillin, streptomycin, polymyxin, chloromycetin, terramycin, sulfaguanidine, and also aureomycin. In all these recent experiments we used younger rats, with an average weight of 50-54 grams, in contrast to the rats used in first studies with an average weight of 138 gm. The survival time of the rats fed the basal experimental necrogenic diet was considerably reduced, from an average of 141 days in the previous observations, to 34-43 days. This finding may be related to the difference in the initial weights: older, heavier rats with their high initial vitamin E stocks will support a more prolonged protection, in contrast to younger animals with their smaller vitamin E reserves

Not only control animals but also the experimental rats receiving supplements of aureomycin to their basal diet showed a reduced survival time (average 110 days) when compared with that of the animals in the previous study.

Among the antimicrobial agents tested, polymyxin, penicillin and chloromycetin were inactive, sulfaguanidine showed only slight indication of a positive effect, whereas streptomycin and terramycin — in this order — were definitely protective, but not as effective as aureomycin

Some of the results obtained are summarized in Figures 3 and 4. As seen from the data in Figure 3 supplements of B_{12} had no effect on the production of dietary massive necrosis, regardless of whether it was added to the basal diet, or the basal diet already supplemented with aureomycin. Thus the effect of aureomycin could not be due to "contamination" with B_{12}

A dilated cecum was often seen in rats receiving supplements of aureomycin or terramycin to the basal yeast diet. The distention of the large intestine was especially pronounced in rats receiving terramycin. Four animals in this group appeared to have died from volvulus of the colon without signs of simultaneous hepatic necrosis All other rats, control and experimental, which died during the course of the experiments have shown microscopic and macroscopic evidence of massive hemorrhagic necrosis of the liver

All the antimicrobial agents used have stimulated, during the first four weeks of the experiments, gain in weight of the animals This growth-stimulation was just as marked with penicillin, chloromycetin

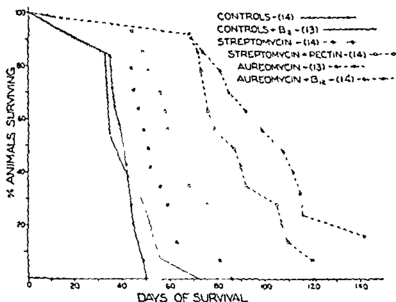


FIGURE 3

Effect of various antibiotics in delaying onset of massive hepatic necrosis in rats fed a necrogenic yeast diet

and polymyxin, which have no, and sulfaguanidine which has barely any, beneficial effect on hepatic necrosis, as with streptomycin, terramycin and aureomycin, which – in this order – were found significantly beneficial in delaying the production of massive hepatic necrosis

Hartroft. Were the animals fed *ad libitum*?

Gyorgy: No, they received uniformly about 8 grams per day The difference in growth stimulation could not be correlated with the average daily food intake

For all practical purposes, ingested streptomycin is not absorbed from the intestinal tract Thus, its positive effect speaks further in favor of the intestinal flora as the site of action Combination with pectin increases the sterilizing effect of streptomycin in the intestine(12). Such combination has been found more effective in delaying massive hepatic necrosis than streptomycin alone (Figure 3). If we assume that antimicrobial agents act through the intestinal flora the quantitative differences in their activity must then be due

Davies: Do you happen to know how often you can get positive blood cultures from the portal vein?

György: In humans?

Davies: Human or rats, because in enteric or other conditions one can often see bacteria in the biopsies inside the sinusoids.

Best: I know some data not yet published where the blood culture became positive after complete ligation of the hepatic artery.

György: In dogs?

Best: Yes.

Gyorgy: Dogs have bacteria in the liver.

Watson: Don't you think that the possible effect of aureomycin on the cellular metabolism of the liver should also be considered?

György: I very seriously entertain such possibility. It is conceivable and perhaps even probable that aureomycin may exert its effect in two directions: 1) via the intestinal flora and 2) direct on the liver cell, or systemically.

Handler: Sulfasuxadine in our hands some years ago prevented choline deficiency cirrhosis.

Gyorgy: We found the same protective effect for thiouracil and propyl-thiouracil. All these substances, including sulfa drugs, may act through the suppression of thyroid activity.

Hartroft: Is the deficiency of vitamin E in the diets used in these experiments concerning hepatic necrosis sufficiently great that testicular atrophy occurs in mature animals or gonadal development fails in young rats?

György. Yes.

Hartroft: When aureomycin is added to the diet, does testicular growth proceed normally or not?

György. They don't develop. I am working now with immature rats The testes do not mature in either group, with or without aureomycin.

Madden: The growth rate is subnormal, less than a gram a day with the aureomycin?

Gyorgy. The diet is a poor diet, especially with regard to protein-intake

Handler: I would like to make a rather assorted comment on the pieces of Dr. György's story. We entered this field belatedly and for reasons which are irrelevant here. But, we have also given B_{12} to young rats on the sort of diets we have been using for choline [illegible]

and fibrotic livers. B_{12} was entirely without effect on either syndrome nor did it even prolong the rats' lives. Since this is on a very low protein diet, this may be pertinent to your considerations concerning the activity of the World Health Organization. One should know whether, on a really low protein diet, B_{12} can really help. These experiments merely open the question.

Best: I think that raises a very interesting question, whether the best experiment would be to give B_{12} alone or try to improve the protein at the same time as you give the B_{12}

Gyorgy. Soya bean, corn meal in pigs or chicks or other animals will promote normal growth when supplemented with B_{12}. Both, beans and corn meal, or similar vegetable proteins, are available in Jamaica There would be no need for importing animal protein, which – as a matter of fact – could not be made available for 1500 million people or more in all those parts of the world where deficiency in animal protein exists Admittedly, the mixture of vegetable proteins to be used should contain all essential amino acids, at least up to the minimum requirement. Analysis of the food to be used will give us the necessary answer.

Best. I think Dr Davies may know The Government in South Africa had before it a recommendation for the introduction of soya beans, it was going to buy them or try to raise them, wasn't that the case?

Davies The Government tried to introduce it in the diet of Uganda Africans but they did not take to it very kindly There you come up against the story of food prejudices.

Handler There are two other additions to this discussion which may be of interest Some of you may remember that Dr. Bernheim and I have been interested in the correlation between the fact that the guinea pig liver contains no choline oxidase and the observation that this species does not develop fatty livers when on choline-deficient rations. Our simple thought was that B_{12} might serve as a choline oxidase inhibitor and thus, in this sense, rats would beh-

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Handler: I would like to make a rather assorted comment on the pieces of Dr. Gyorgy's story. We entered this field belatedly and for reasons which are irrelevant here. But, we have also given B_{12} to young rats on the sort of diets we have been using for choline and cystine deficiency, *viz.*, 5% casein, a great deal of carbohydrate and very little fat. By including choline or cystine one can readily obtain cystine deficiency hepatic necrosis or choline deficiency fatty and fibrotic livers. B_{12} was entirely without effect on either syndrome nor did it even prolong the rats' lives Since this is on a very low protein diet, this may be pertinent to your considerations concerning the activity of the World Health Organization. One should know whether, on a really low protein diet, B_{12} can really help. These experiments merely open the question.

Best: I think that raises a very interesting question, whether the best experiment would be to give B_{12} alone or try to improve the protein at the same time as you give the B_{12}

Gyorgy. Soya bean, corn meal in pigs or chicks or other animals will promote normal growth when supplemented with B_{12}. Both, beans and corn meal, or similar vegetable proteins, are available in Jamaica There would be no need for importing animal protein, which – as a matter of fact – could not be made available for 1500 million people or more in all those parts of the world where deficiency in animal protein exists Admittedly, the mixture of vegetable proteins to be used should contain all essential amino acids, at least up to the minimum requirement Analysis of the food to be used will give us the necessary answer.

Best I think Dr Davies may know. The Government in South Africa had before it a recommendation for the introduction of soya beans, it was going to buy them or try to raise them, wasn't that the case?

Davies The Government tried to introduce it in the diet of Uganda Africans but they did not take to it very kindly There you come up against the story of food prejudices

Handler There are two other additions to this discussion which may be of interest Some of you may remember that Dr. Bernheim and I have been interested in the correlation between the fact that the guinea pig liver contains no choline oxidase and the observation that this species does not develop fatty livers when on choline-deficient rations Our simple thought was that B_{12} might serve as a choline oxidase inhibitor and thus, in this sense, rats would behave

Davies: Do you happen to know how often you can get positive blood cultures from the portal vein?

György: In humans?

Davies: Human or rats, because in enteric or other conditions one can often see bacteria in the biopsies inside the sinusoids.

Best: I know some data not yet published where the blood culture became positive after complete ligation of the hepatic artery.

György: In dogs?

Best: Yes.

György: Dogs have bacteria in the liver.

Watson: Don't you think that the possible effect of aureomycin on the cellular metabolism of the liver should also be considered?

György: I very seriously entertain such possibility. It is conceivable and perhaps even probable that aureomycin may exert its effect in two directions: 1) via the intestinal flora and 2) direct on the liver cell, or systemically

Handler: Sulfasuxadine in our hands some years ago prevented choline deficiency cirrhosis.

György: We found the same protective effect for thiouracil and propyl-thiouracil. All these substances, including sulfa drugs, may act through the suppression of thyroid activity.

Hartroft: Is the deficiency of vitamin E in the diets used in these experiments concerning hepatic necrosis sufficiently great that testicular atrophy occurs in mature animals or gonadal development fails in young rats?

György: Yes.

Hartroft: When aureomycin is added to the diet, does testicular growth proceed normally or not?

György: They don't develop. I am working now with immature rats. The testes do not mature in either group, with or without aureomycin.

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György: The diet is a poor diet, especially with regard to protein-intake.

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With respect to the metabolic mechanisms involved, this is an almost independent story which had its beginnings completely apart from work with B_{12}. In essence, the picture can be stated thus: In the animal economy there exist mechanisms whereby preformed methyl groups can be oxidized to intermediates like formaldehyde and formate. These can be reduced back to methyl groups or to whatever is necessary for the synthesis of serine, glycine and purines The earliest pertinent observations were made by Dakin(26) before World War I when he studied the excretion of formate by man as a function of diet and he actually concluded that formate was an end product of protein metabolism. The first demonstration of the formation of a C_1 compound from a normal metabolite was that by Drs. Handler, Bernheim, and Klein(27) who demonstrated the existence of an enzyme system in liver which oxidatively demethylated sarcosine to glycine and formaldehyde. This has been beautifully substantiated by Mackenzie(28) and du Vigneaud who have also found that a large portion of the methionine or choline methyl groups soon appear as expired CO_2. The intermediate details have been considered in only a few laboratories. At Columbia, Shemin(29) observed that serine was converted to glycine and suggested formate as the other product in 1946. In Berkeley, Greenberg and his students have since found. *1.* labeled formate in the medium after incubating liver slices with methylene labeled glycine(30); *2.* α and β labeled serine in the protein of rat liver homogenates after incubation with methylene labeled glycine(30); *3.* $HC^{14}OOH$ and $HC^{14}HO$ after incubation of liver with β-labeled serine, methylene labeled glycine, methyl labeled methionine and choline(31). In Cleveland, Sakami found: *1.* carboxyl and β-labeled serine in rat tissue protein after giving carboxyl labeled glycine and labeled formate respectively(32); *2.* α-β labeled serine in rat tissues after feeding methylene labeled glycine(33); *3* β-labeled serine after feeding methyl labeled choline(34); *4.* methyl labeled tissue choline and methionine after repeated administration of $HC^{14}OOH$ (35), and *5* labeled serine even after giving methyl labeled acetone to rats(36). Evidence has been offered that it is formaldehyde rather than formic acid which is the precursor of the β-carbon of serine and the methyl groups of choline and methionine(37). Another line of evidence is that relating to purine synthesis. Thus, Buchanan has stated that formate carbon is incorporated into positions 2 and 8 of uric acid(38), Barker found that the α-carbon of glycine also entered these positions(39), Greenberg(40) found that pigeon liver homogenates utilized formate for the synthesis of hypoxanthine

nd Marsh(41) reported that pigeons incorporated the carbon of ormic acid into nucleic acid guanine and adenine Each of these tudies involved the use of isotopic carbon Thus, the old labile nethyl pool has been considerably expanded, or perhaps, lost its neaning entirely, if ever it had one. There is no clean cut evidence of the site of B_{12} action in all this but Lardy(42) has reported a ignificant decrease in the ability of folic acid deficient rats to fix abeled formate and Sprinson(43) has reported a marked decrease n the rate of conversion of labeled serine to glycine in similar nimals It should be noted that neither group reported any non-pecific controls but their findings seem compatible with the whole picture Two criticisms may be made of these studies in general First, in most instances the specific activity of the original material nas been many orders of magnitude greater than that isolated. One cannot be too careful to avoid the danger of a small amount of hot contaminant in such cases, particularly when such minute amounts of material are involved. Second, even if this danger has been avoided, as a *net* phenomenon, of what real metabolic and nutritional significance is all this? Certainly, it provides a mechanism for the synthesis of dietary nonessentials like glycine, serine and purines, but to what extent may one really dispense with dietary methyl groups? While the analogy may be dangerous, it may also be well to note that CO_2 fixation in mammals is now well established, and the reactions involved appear to be remarkably similar to the pathways of photosynthesis, but we must, nevertheless, continue to consume 2,000 calories and some reasonable amount of protein per day. Nutritionally the problem becomes, "What is a reasonable protein consumption when B_{12} is supplied?" And from this same standpoint, one cannot help but be intrigued with the possibilities of methanol as a growth factor and lipotropic agent!

Best: I think this summary is extremely valuable for those of us who want to go back and do more experiments

Schiff I have a clinical observation that may be of some interest to Dr Gyorgy We recently had a young woman with phosphorus poisoning to whom we gave one milligram (1000 gamma) vitamin B_{12}, one milligram per day for five or seven days She died at the end of two weeks At autopsy her liver showed fatty vacuolization and considerable necrosis I mention it for whatever it might be worth

Gyorgy: B_{12} has no effect on dietary hepatic necrosis According to Dr. Popper it may have a slight beneficial effect on cellular

Handler: If you start with the larger rats, the most obvious phenomenon is their markedly increased survival time. In fact a large proportion survived the entire 6-month experimental period And a great fraction of these show nothing but exceedingly fatty livers. A small fraction could be described as "cirrhotic."

With respect to cystine deficiency, then, the picture is much like that which Dr. Gyorgy suggested. The young rats died in about one month while the older ones survived very much longer. In the young rats we find the now classical massive necrosis, in the older rats little or nothing. What bothers me is that the cystine-deficient young rats who do not show hepatic necrosis die after the same time on the diet as do those with necrosis. Nor have we found any other lesion to account for their early deaths.

Gyorgy: For the record, I have now succeeded in securing an American type of yeast which produces massive necrosis It is not a prerogative of the British.

Watson: Dr. Handler, what sort of diet do you use?

Handler: Five percent casein, a mixture of sucrose and starch and about 20 percent fat.

Watson: What percent of the rats get massive necrosis on that diet?

Handler: A bit better than 50%, I think.

Hoffbauer: I should like to ask, Dr. Handler, if the character of the dietary fat varied.

Handler It was somewhat different in the choline- and the cystine-deficient diets

Hoffbauer: What is the nature?

Handler A mixture of cottonseed oil and commercial Jewell shortening

Watson: No yeast?

Handler No yeast.

Fremont-Smith: I am very grateful for this particular illustration of what I said at the very beginning and that is the response of the organism is as much determined by the state of the organism as by the nature of the stimulus.

Shorr: Do I remember Dr. Gyorgy saying last year that he had a strain of rats particularly susceptible to cirrhosis?

György. Yes, that was the Fisher strain. With this strain of rats I obtained the most pronounced and uniform fatty liver on lipotropic diet The individual variations were negligible. In addition, as Dr. Tarver found for the same strain, liver slices obtained from Fisher rats do not take up methionine as readily as liver cells of other strains

Tarver: The uptake of methionine by slices of liver taken from a Fisher strain rat is generally found to be less than what we have found with other strains Presumably, therefore, its rate of protein synthesis, or turnover, is less.

Gyorgy· Rats of the Fisher strain show this poor methionine uptake and the same strain is especially sensitive to an alipotropic diet

Shorr: Where is the Fisher strain obtained from?

Gyorgy: From Dr W. F. Dunning, Wayne University College of Medicine, Detroit.

Watson· Dr Gyorgy, am I mistaken in thinking that you had a rather low incidence of massive necrosis except when you used British yeast?

Gyorgy: Never higher than 40 percent.

Watson Do you know why there was the difference?

Gyorgy: No, not yet

Shorr. I had one experience with yeast which suggests that there may be differences between English and American strains I had bad luck with attempting to isolate co-enzyme I from American yeast during a summer in England Dr Green was good enough to let me carry out a similar preparation with British yeast in the Cambridge Laboratory of Hopkins and I obtained a wonderful yield, using exactly the same methods that had proved unsuccessful with American yeast

Handler· The variable properties of yeast have played quite a role in the history of biochemistry. Pasteur, about 25 years before the brothers Buchner, made an extract of brewers yeast much in the same manner as the Buchners in a deliberate attempt to prepare a cell-free extract which would ferment. But he got his yeast from

3. HALL, C. A., and DRILL, V. A.: Lipotropic effect of liver extract on dietary hepatic injury in rats. *Proc. Soc. Exper. Biol. & Med* 69, 3 (1948).

4. DRILL, V. A., and MCCORMICK, H. M.: Lipotropic effects of vitamin B_{12} concentrate. *Proc. Soc Exper. Biol & Med.* 72, 388 (1949).

5. GYORGY, P., and ROSE, C. S.: Effect of vitamin B_{12} on experimental hepatic injury. *Proc. Soc. Exper. Biol & Med.* 73, 372 (1950).

6. SCHAEFER, A. E., SALMON, W. D., and STRENGTH, D. R.: Interrelationship of vitamin B_{12} and choline. I. Effect on hemorrhagic kidney syndrome in the rat. *Proc. Soc. Exper. Biol. & Med.* 71, 193 (1949)

7. ————, ————, and ————: Role of vitamin B_{12} and methyl donors in lipotropism and transmethylation in the rat and chick. *Federation Proc* 9, 369 (1950)

8. STEKOL, J. A., *et al.*, Sulfur-containing amino acids in growth with a 'labile methyl'-free diet containing vitamin B_{12}. *Federation Proc* 9, 234 (1950).

9. WELCH, A. D., and SAKAMI, W.: Synthesis of labile methyl groups by animal tissues *in vivo* and *in vitro*. *Federation Proc* 9, 245 (1950)

10. BEAN, W. B., FRANKLIN, M., and SAHS, A. L.: An effect of vitamin B_{12} on pain in nutritional neuropathy *Am. J. Med. Sc* 220, 431 (1950).

11. *Liver Injury* HOFFBAUER, F. W., Editor Trans. Eighth Conf New York Josiah Macy, Jr Foundation, 1949 (p. 56).

12. PULASKI, E. J., and CONNELL, J. F., JR: Control of peritoneal infection in gastrointestinal surgery *Bull U. S. Army Med. Dept* 9, 265 (1949)

13. SBOROV, V. M., JAY, A. R., and WATSON, C. J.: The effect of aureomycin on urobilinogen formation and the fecal flora *J. Lab & Clin Med* 37, 52 (1951)

14. HERVE, A., and BACQ, Z. M. Cyanure et dose léthale de rayons X *Compt rend Soc. de biol* 143, 881 (1949).

15. PATT, H. M., *et al* Cysteine protection against X irradiation *Science* 110, 213 (1949)

16. HOWLAND, J. W., FURTH, F., and COULTER, M. Effect of aureomycin and antibiotics on whole body irradiation *Federation Proc* 9, 334 (1950)

17. DU VIGNEAUD, V., *et al* Effect of choline on the ability of homocystine to replace methionine in the diet *J. Biol. Chem.* 131, 57 (1939).

18. HANDLER, P. Factors affecting the occurrence of renal lesions due to choline deficiency *J. Nutrition* 31, 621 (1946).

19. DU VIGNEAUD, V., *et al* Synthesis of labile methyl groups in the white rat *J. Biol Chem* 159, 755 (1945)

20 BENNETT, M. A, MEDES, G, and TOENNIES, G · Growth of albino rats on a choline-free diet in which homocystine is the only sulfur-containing amino acid. *Growth* 8, *59 (1944)*

21 SCHAEFER, A E, SALMON, W. D, and STRENGTH, D R · Interrelationship of vitamin B_{12} and choline I. Effect on hemorrhagic kidney syndrome in the rat *Proc. Soc. Exper. Biol & Med* 71, *193 (1949)*

22 GILLIS, M M, and NORRIS, L C · Effect of the animal protein factor on the requirement for methylating compounds. *J. Biol Chem* 179, *487 (1949)*

23 JUKES, T H, STOKSTAD, E. L R, and BROQUIST, H. P · Effect of vitamin B_{12} on the response to homocystine in chicks *Arch Biochem* 25, *453 (1950)*

24 STEKOL, J A, and WEISS, K · Vitamin B_{12} and growth of rats on diets free of methionine and choline *J. Biol. Chem* 186, *383 (1950)*

25. GYORGY, P, and ROSE, C S · Effect of vitamin B_{12} on experimental hepatic injury *Proc Soc. Exper Biol & Med* 73, *372 (1950).*

26 DAKIN, H D, JANNEY, N W., and WAKEMAN, A J · Studies on the conditions affecting the formation and excretion of formic acid *J Biol Chem* 14, *341 (1913)*

27 HANDLER, J, BERNHEIM, M L C, and KLEIN, J R · Oxidative demethylation of sarcosine to glycine *J Biol. Chem* 138, *211 (1941)*

28 MACKENZIE, C G, and DU VIGNEAUD, V Formation of formaldehyde in the biological oxidation of the methyl group of sarcosine *Federation Proc* 8, *223 (1949)*

29 SHEMIN, D The biological conversion of *l*-serine to glycine *J Biol Chem* 162, *297 (1946)*

30 SIEKEVITZ, P, and GREENBERG, D M The biological formation of serine from glycine *J Biol Chem* 180, *845 (1949)*

31 SIEKEVITZ, P, and GREENBERG, D M Biological formation of formate and formaldehyde from methyl compounds in liver slices *Federation Proc* 9, *227 (1950)*

32 SAKAMI, W The conversion of formate and glycine to serine and glycogen in the intact rat *J Biol Chem* 176, *995 (1949)*

33 SAKAMI, W The conversion of glycine into serine in the intact rat *J Biol Chem* 178, *519 (1949)*

34 SAKAMI, W The formation of the β carbon of serine from choline methyl groups *J Biol Chem* 179, *495 (1949)*

35 WELCH, A D, and SAKAMI, W Synthesis of labile methyl groups by animal tissues *in vivo* and *in vitro* *Federation Proc 9, 245 (1950)*

36 SAKAMI, W: Acetone metabolism in the intact rat *Federation Proc. 9, 222 (1950)*